*UNIVERSITATEA DIN CRAIOVA*
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***Seria:*** ✓*Biologie*
✓*Horticultură*
✓*Tehnologia prelucrării produselor agricole*
✓*Ingineria mediului*

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# ASPECTS OF COENOLOGICAL INTEGRATION OF *SAPONARIA PUMILIO* (L.) FENZL. EX. A. BRAUN IN *POTENTILLO CHRYSOCRASPEDAE–FESTUCETUM AIROIDIS ASSOCIATION*

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**Key words**: *Saponaria pumilio*, *plant associations, similarity*.

## ABSTRACT

*Saponaria pumilio is a tertiary relict and a rare plant in Romania's flora. In 1993, Stancu, Alexiu and Boşcaiu have integrated the species in Potentillo chrysocraspedae–Festucetum airoidis association. In this paper, we present the coenotaxonomical integration of Saponaria pumilio species in some phytocoenoses and describe the association in terms of taxonomic, of the bioforms, geoelements, caryological, ecological index. We characterized the Potentillo chrysocraspedae–Festucetum airoidis association based on some phytocoenological investigations from Meridional Carpathians. This study revealed some common and differential features of this association, from different places, being drown a similarity dendrogram of the analyzed relevés.*

## INTRODUCTION

*Saponaria pumilio* is an herbaceous species from *Caryophyllaceae* family. It is a perennial herb, with shorter stems, forming dense, large cushions. Flowers are large and pink with a tubular calyx. Seeds are 1.0–1.5 mm diameter and have no capacity for dispersal over long distances. Generally, the species vegetate on skeletal, siliceous soils. It is taxonomically isolated and occurs dispersed in the Eastern Alps (Italy, Austria) and Romanian Carpathians (Iezer-Păpuşa and Făgăraş Massif) (Ciocârlan, 2000; Stancu et al, 1993).

The distribution of *Potentillo chrysocraspedae–Festucetum airoidis* association is between 1750 m and 2244 m altitude. This association forms compact layers on gentle slopes from alpine and sub-alpine regions and vegetates on less deep soils, from very acid to slightly acid, often rich in humus, formed on the crystalline or calcareous substrate. The association *Potentillo chrysocraspedae-Festucetum airoides* Boşcaiu 1971 is classified in the class *Juncetea trifidi* Klika et Hadac 1944, order *Caricetalia curvulae* Br.-Bl. 1926, alliance *Caricion curvulae* Br.-Bl. 1925 (Zamfirescu et al., 2007; Alexiu, 1998; Drăgulescu, 2003).

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## MATERIAL AND METHODS

For the relevés study, we used the methods of the Central-European school of Zürich-Montpellier, elaborated by J. Braun-Blanqe. For the synthetic phytocoenological table were used personal investigations and works of some authors (Alexiu, 1998; Boşcaiu, 1971; Neblea, 2008; Stancu, 2005; Drăgulescu, 2003).

The plant nomenclature follows Ciocârlan, 2009.

The synthetic phytocoenological table of *Potentillo chrysocraspedae–Festucetum airoidis* association (Table 1) inserts list of species from identified coenoses in Iezer-Păpuşa Massif (Alexiu, 1998; Andronescu, 2011), Leaota (Neblea, 2006), Ţarcu, Godeanu, Cernei (Boşcaiu, 1971), Cindrel-Valea Sadului (Drăgulescu, 1995) and Râiosu-Buda (Stancu, 2005). In the left side of the synthetic table (Table 1) are included the phytocoenological parameters of bioforms, geoelements and caryologic elements; the right side includes five columns which contain the constant (K) of each species, that correspond to different mountains. The similarity of relevés was been assessed by using a statistical processing program (BioDiversity Pro).

## RESULTS AND DISCUSSIONS

The *Potentillo chrysocraspedae-Festucetum airoidis* association classifies in the *Juncetea trifidi* class, *Caricetalia curvulae* order, *Caricion curvulae* alliance. It forms oligotrophic alpine meadows on highly acidic soils. The association is present in almost all Carpathians Massive. The presence of *Potentilla ternata*, along with other geographical differential species (*Poa media*, *Viola declinata*, *Campanula serrata* and *Campanula patula ssp. abietina*) shows the affiliation of this association to Carpathian-Balkan area. *Festuca supina* meadows are widespread in alpine and sub-alpine regions and characterized by a remarkable homogeneity structure. Because is characterized by a xeric regime, the chionofile species are missing from *Potentillo chrysocraspedae-Festucetum airoidis* association.

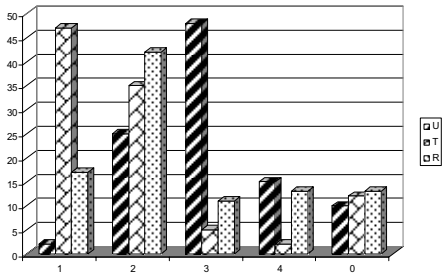


Figure 1. Ecological index of *Potentillo chrysocraspedae-Festucetum airoidis* association

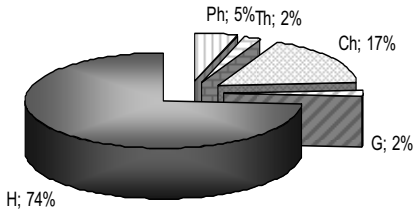


Figure 2. Bioforms of the *Potentillo chrysocraspedae-Festucetum airoidis* association

The phytocoenoses of the associations are mesophilic ($U_{3-3,5}$= 48.27 %), cryophilic to microtherm ($T_{1-1,5}$=47.12 % ; $T_{2-2,5}$= 34.48 %) and acidophilous ($R_2$=42.52% ) (Figure 1). The index of bioforms reveals a high frequency of the hemicryptophytes (74%), followed by a significant percentage of camephytes (17%) (Figure 2).

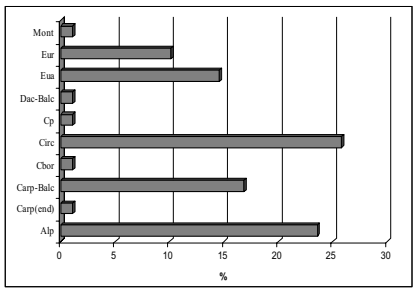


Figure 3. Phytogeographic elements of *Potentillo chrysocraspedae-Festucetum airoidis* association

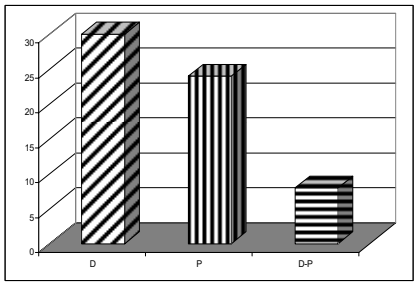


Figure 4. Caryological index of the *Potentillochrysocraspedae– Festucetum airoidis* association

With regard to phytogeographic elements, predominates the Circumpolar (25.84 %) and Alpic species (23.59 %), followed by the Carpathian-Balkan (16.85 %), Eurasian (14.6 %), European (10.11 %) (Figure 3). In the caryological spectrum are included diploid (31.18 %), polyploid (29.03 %) and diplo-polyploid (12.9 %) species and the index of ratio is 1.25 (Figure 4).

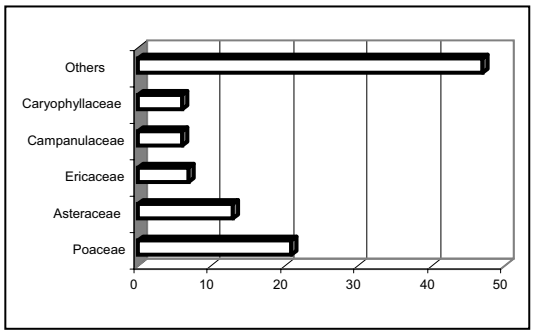


Figure 5. Taxonomic index of the *Potentillo chrysocraspedae–Festucetum airoidis* association

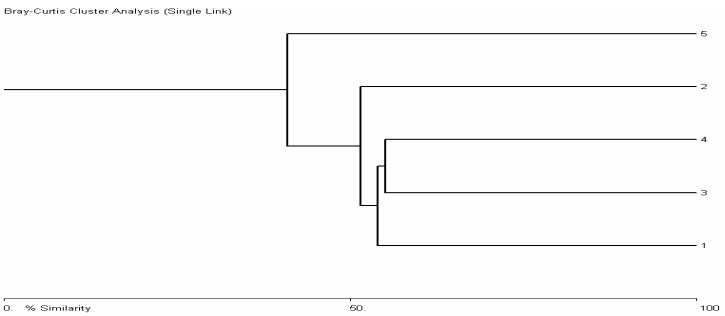


Figure 6. Similarity dendrogram of the phytocoenoses of *Potentillo chrysocraspedae-Festucetum airoidis* association

The specific diversity of the association lies in the presence of the 87 cormophitic taxa that are integrated in 27 families, from which: Poaceae (20. 68 %), Asteraceae (12.64 %), Ericaceae (6.89 %), Campanulaceae (5.7 %) and Caryophyllaceae (5.7 %) (Figure 6).

The relevés similarity dendrogram (Figure 6) results from the similarity Jaccard index calculation. This highlights the homogeneity of the phytocoenoses identified in Cindrel, Țarcu, Godeanu and Cernei. *Saponaria pumilio* is present only in the phytocoenoses from Iezer-Păpușa and Făgăraș Mountains.

## CONCLUSIONS

The association *Potentillo chrysocraspedae-Festucetum airoidis* Boşcaiu 1971 is classified in the class *Juncetea trifidi* Klika et Hadac 1944, order *Caricetalia curvulae* Br.-Bl. 1926, alliance *Caricion curvulae* Br.-Bl. 1925.

The association phytocoenoses bioforms are frequently hemicryptophytes (74%).

With regard to phytogeographic elements, predominates the Circumpolar (25.84 %) and Alpic species (23.59 %), followed by the Carpathian-Balkan (16.85%) and Eurasian (14.6 %).

In terms of the ecological preferences, the phytocoenoses of the *Potentillo chrysocraspedae–Festucetum airoidis* associations are mesophilic, cryophilic and acidophilous. Diploids index is higher than one (1.25) because the number of diploid species is higher than the polyploid one.

The phytocoenological investigation, based on the relevés analysis, revealed the presence of *Saponaria pumilio* only in the phytocoenoses from Iezer-Păpușa and Făgăraș Mountains.

## ACKNOWLEDGEMENTS

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Table 1

*Potentillo chrysocraspedae–Festucetum airoidis* Boşcaiu 1971 association

| | **Place of the relevés** | | | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|
| | **Altitude (m x 10)** | | | 190/ 240 | 160/ 180 | 155/ 216 | 176/ 221 | 180/ 200 |
| | **Number of relevés** | | | 10 | 7 | 20 | 21 | 10 |
| | | | **Char.ass.** | | | | | |
| H | Eua-Arc-Alp | DP | *Festuca airoides* | V | V | V | - | V |
| H | Carp-Balc | - | *Potentilla ternata* | V | V | V | V | V |
| | | | **Carcion et Caricetalia curvulae** | | | | | |
| Ch | Alp-Carp-Eur | P | *Primula minima* | IV | III | II | II | IV |
| H | Alp-Carp-Balc | P | *Phyteuma confusum* | IV | - | III | IV | III |
| H | Alp-Eur | DP | *Agrostis rupestris* | IV | III | V | II | IV |
| H | Circ | - | *Luzula spicata* | - | - | I | I | III |
| H | Alp-Carp | D | *Campanula alpina* | I | I | - | - | III |
| H | Alp-Eur | P | *Carex curvula* | I | - | I | - | - |
| H | Carp-Balc | P | *Senecio carpaticus* | - | - | I | - | I |
| Th | Eua | - | *Euphrasia minima* | - | - | I | - | - |
| H | End-Carp | D | *Helictotrichon decorum* | - | - | I | I | - |
| H | Circ | - | *Anthoxanthum alpinum* | - | - | I | - | - |
| | | | **Juncetea trifidi** | | | | | |
| H | Circ-Arc-Alp | D | *Juncus trifidus* | IV | III | I | I | IV |
| H | Carp-Balc | D | *Poa media* | III | - | - | I | - |
| H | Alp-Carp | DP | *Armeria alpina* | - | II | - | - | - |
| Ch | Alp-Eur | D | *Minuartia sedoides* | - | III | - | - | - |
| | | | **Loiseleurio-Vaccinion** | | | | | - |
| Ch | Circ-Arc-Alp | D | *Vaccinium uliginosum* | I | - | I | I | - |
| Ch | Circ-Arc-Alp | D | *Loiseleuria procumbens* | I | - | I | I | - |
| | | | **Potentillo-Nardion** | | | | | |
| H | Alp-Eur | P | *Geum montanum* | III | III | IV | III | - |
| H | Eua (Circ.) | D | *Nardus stricta* | IV | III | I | III | - |
| TH | Carp-Balc | P | *Campanula patula* | III | III | I | - | II |
| H | Eur | P | *Festuca nigrescens* | II | - | - | - | - |
| H | Circ-Alp | P | *Phleum alpinum* | II | III | - | III | - |
| H | Alp-Eur | D | *Ligusticum mutellina* | I | III | III | III | IV |
| H | Alp-Eur | P | *Homogyne alpina* | I | II | I | II | - |
| H | End-Carp | D | *Campanula serrata* | - | III | - | I | - |
| H | Carp-Balc | P | *Viola declinata* | - | III | - | I | - |
| H | Eua | P | *Antennaria dioica* | - | III | I | II | - |
| | | | **Salicetalia herbaceae** | | | | | |
| Ch | Alp-Eur | D | Sedum alpestre | II | - | I | I | - |
| | | | **Varia syntaxa** | | | | | |
| Ch | Alp-Carp | P | *Saponaria pumilio* | III | - | - | - | IV |
| H | Alp-Carp | D | *Oreochloa disticha* | II | - | I | I | IV |
| nPh | Alp-Carp | - | *Rhododendron myrtifolium* | II | - | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mPh | Arc-Alp | D | *Juniperus communis* | II | I | - | III | - |
| Ph | Circ | D | *Vaccinium vitis-idaea* | I | III | II | II | - |
| Ph | Circ | D | *Vaccinium myrtillus* | - | II | I | III | - |
| H | Circ | DP | *Deschampsia flexuosa* | I | IV | I | I | - |
| H | Alp-Carp-Balc | - | *Anthemis carpatica* | I | - | - | - | - |
| nPh | Carp-Balc | P | *Bruckenthalia spiculifolia* | I | III | - | I | - |
| Ch | Circ-Arc-Alp | D | *Silene acaulis* | I | - | - | - | - |
| H | Carp(end) | - | *Chrysosplenium alpinum* | I | - | I | - | - |
| H | Circ-Arc-Alp | DP | *Veronica alpina* | I | - | I | - | - |
| Ch | Circ-Arc-Alp | P | *Cerastium cerastoides* | I | II | II | - | - |
| G | Circ-Arc-Alp | P | *Polygonum viviparum* | I | - | I | - | - |
| H | Alp-Carp | D | *Pulsatilla alba* | - | I | I | I | III |
| H | Circ-Arct | D | *Pedicularis verticillata* | - | III | III | - | - |
| H | Eua | D | *Centaurea nervosa* | - | III | - | - | - |
| H | Eua | P | *Trifolium repens* | - | III | - | I | - |
| H | Circ (Arct-Alp) | - | *Hieracium alpinum* | - | - | I | III | III |
| H | Eua | DP | *Anthoxanthum odoratum* | - | - | - | III | - |
| H | Circ | DP | *Festuca rubra* | - | - | - | II | - |
| H | Circ-Arct-Alp | P | *Gnaphalium supinum* | - | - | III | II | - |
| H | Subarct.-Alp | P | *Luzula sudetica* | I | - | I | II | - |
| H | Alp-Eur | P | *Soldanella pusilla* | - | - | I | II | - |
| H | Alp-Carp-Balc | P | *Soldanella hungarica* | - | - | - | I | - |
| H | Eua-Arct-Alp | D | *Arenaria biflora* | - | - | - | I | - |
| H | Eua-Arct-Alp | - | *Ranunculus montanus* | - | - | - | I | - |
| H | Alp-Carp | - | *Aconitum tauricum* | - | - | - | I | - |
| H | Eur | P | *Alchemilla vulgaris* | - | - | - | I | - |
| H | Carp-Balc | D | *Plantago gentianoides* | - | - | - | I | - |
| H | Circ | D | *Eriophorum vaginatum* | - | - | - | I | - |
| H/G | Circ | D | *Oxalis acetosella* | - | - | - | I | - |
| Ch | Eur(mont.) | - | *Thymus pulegioides* | - | - | - | I | - |
| Ch | Eur | - | *Polytrichum juniperinum* | I | - | II | III | - |
| G | Dac-Balc | P | *Carex nigra* | - | - | - | I | - |
| H | Circ | - | *Carex atrata* | - | - | - | I | - |
| H | Eua-Alp | - | *Taraxacum alpinum* | - | - | - | I | - |
| H | Cosm | - | *Veronica serpyllifolia* | - | - | - | I | - |
| G | Eur (mont) | P | *Pseudorchis albida* | - | - | - | I | - |
| Ph | Eur | D | *Picea abies* | - | - | - | I | - |
| H | Carp-Balc | - | *Senecio glaberrimus* | I | - | - | - | - |
| H | Alp-Carp | P | *Senecio carniolicus* | I | - | - | - | - |
| G | Eua(Circ) | - | *Anthoxanthum alpinum* | I | - | - | - | - |
| H | Alp-Carp-Balc | - | *Ranunculus crenatus* | - | - | I | - | - |
| H | Euc | D | *Luzula luzuloides* | - | - | I | - | - |
| Ch | Circ | D | *Selaginella selaginoides* | - | - | I | - | - |
| H | Circ | DP | *Parnassia palustris* | - | - | I | - | - |
| Ch | Eua(Circ) | - | *Cerastium alpinum* | - | - | I | - | - |
| H | Alp-Carp | D | *Avenula versicolor* | - | - | - | II | II |
| H | Circ-Arc-Alp | DP | *Poa alpina* | - | - | I | III | III |
| H | End-Carp | - | *Dianthus glacialis* | - | - | - | II | II |
| H | Alp-Eur | D | *Tanacetum alpinum* | - | - | - | I | - |
| H | Circ | P | *Luzula multiflora* | - | - | I | - | - |
| Ch | Carp-Balc | D | *Thymus balcanus* | - | - | I | - | - |
| Ch | Balc. | D | *Sesleria bielzii* | - | - | I | - | - |

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# RESEARCH ON SECRETING TISSUE AND THE VOLATILE OIL COMPOSITION OF *ARTEMISIA DRACUNCULUS /ARTEMISIA DRACUNCULUS VAR. PILOSA* SPECIES

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***Key words*:** *volatile oils, phenophase, secreting tissue, Artemisia sp., chromatography*

## ABSTRACT

*The work presents aspects concerning secreting tissues and the composition of volatile oil on the species Artemisia dracunculus and Artemisia dracunculus var. pilosa. The transversal sections through leaf revealed the presence of multicellular secretory hairs and secretory channels. Analysis of volatile oil, extracted from herba in different phenophases, revealed the presence of important chemical compounds, as follows: camphen, eucalyptol, borneol, camphor, at A. dracunculus species, and sabinene, methyl eugenol, gamma-terpinene, terpinene-4 ol, cis-beta-ocimene at A. dracunculus var. pilosa species.*

## INTRODUCTION

*Artemisia* L. (*Asteraceae)* genre comprises about 500 species, it's the largest genre of the *Anthemideae* tribe and one of the largest genres of *Asteraceae* family (Torrell and al., 1999; Bohm and Stuessy, 2001; Torrell and Vallès, 2001; Watson and al., 2002).

Most species belonging to this genre have been classified in the group of medical and aromatic plants, with use in medicine, nutrition and industry. The extracts obtained from *Artemisia dracunculus* species proved to have antiscorbutic, diuretic, digestive, febrifuge, hypnotic and vermifuge action (Burzo et al., 2005; Obolskiy et al., 2011; Teresa et al., 2012), being used in medicine. Leaves and young stems are used in nutrition, to flavor various dishes.

*A. dracunculus* species is described in detail in Romanian Flora, vol IX (Săvulescu et al. 1964), and *A. dracunculus var. pilosa* was identified for the first time in Romania by Ciocârlan (2010) (unpublished data). After Koamarovii (1961) it is distinguished in early vegetation by leaves and stems intensively hairy, being spread in the steppes of Zavoljie region.

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The purpose of the present paper was to identify the secretory tissues and to analysis of volatile oil composition for the species *A. dracunculus* (Fig.1. a ) and *A. dracunculus var. pilosa* (Fig.1.b.).

Fig.1 a) *Artemisia dracunculus* b) *A..dracunculus* var. *pilosa* Krasch, morphological aspects

According to Toma and Ruginã (1998) the leaves of *A. dracunculus* species present at the upper and lower epidermal level multicellular tector, unseried hairs. In the vicinity of conducting fasciculus of mesophyll, there are secretory ducts. Zani et al. (1991) analyzed the volatile oil from *A. dracunculus* plants from France and found that the main substances were: metilcavicol 60-80%, cis/trans ocimene 6-12% and limonene 2-6 %. The main chemical compounds present in the volatile oil extracted from *A. dracunculus* plants colected from Poland were: elemicine 48,78%, sabinene 18,88%, cis-isoelemicine 13,32% and methyl eugenol 7,63% (Kowalski et al., 2007).

## MATERIAL AND METHODS

In order to realize the research it has been used fresh vegetal material and vegetal material preserved alcohol, represented by foliar limb, taken from *A. dracunculus* and *A. dracunculus var. pilosa*.

Conducted sections were examined and photographed under an Optika microscope.

Volatile oils were extracted from herbs, from *Artemisia* plants in different growth stages (blooming, flowering, and exceeded maturity respectively).

The volatile compounds were extracted by hydrodistillation with a Singer-Nickerson apparatus. The separation and identification of components has been carried out using an Agilent gas chromatograph, equipped with quadruple mass spectrometer detector. A capillary column DB-5 (25 m length x 0.25 mm i.d. and 0.25 µm film thickness) and helium as carrier gas were used. The initial oven temperature was 60°C, then rising to 280 °C at a rate of 4°C /min. The NIST spectra bank was used for to identify the volatile compounds, which were verified with the Kovats indices.

## RESULTS AND DISCUSSIONS

Anatomical analyzes conducted on the leaves of *A. dracunculus* species (Fig. 2a) have emphasized the presence of a thick cuticle, a mesophyll of ecvifacial type at the epidermis level and at the shows a thick cuticle, and at the abaxial angle it has been observed a

secretory channel, of large dimensions, surrounded by conducting beams. These findings are consistent with the results of Toma and Rugină (1998).

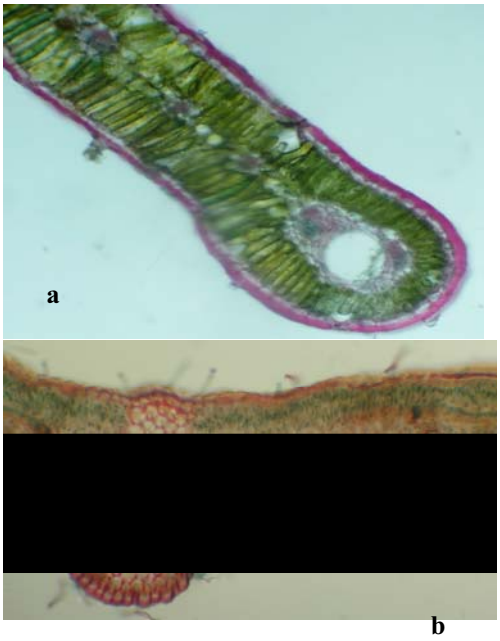


Fig. 2 a-b. Leaf transversal section. *A. dracunculus* / *A. dracunculus var. pilosa*

In the leaves of *A. dracunculus var. pilosa* (fig. 2b) the presence of some cells of irregular forms, with slightly wavy sides was observed. On the outside is the cuticle and both epidermis present isodiametric cells. At the epidermis level, from place to place, there are uniseried multicellular tector hairs and secreting hairs, in a greater number in the depressions of abaxial epidermis and on the adaxial side. The palisade tissue consists of two rows of cells. The conducting fasciculus type is collateral open, with wood facing the adaxial side.

Regarding the analysis of volatile oil extracted from *A. dracunculus* plants and the variety of *A. dracunculus pilosa,* it has been observed a quantitative decrease of compounds from flowering stage to the exceeded maturity one. The results obtained were as follows: sabinene by 1.58%, terpinene-4-ol by 1.75%, methyl eugenol by 1.99%, gamma-terpinene by 2.46% and complete disappearance of cis-beta-ocimene. I was noticed a remarkably increase of the amounts of phenylpropanoids and sesquiterpene compound groups.

The obtained data show remarkable differences in the composition of volatile oil extracted from *A. dracunculus* and *A. dracunculus var. pilosa* (Table 1.).

At the variety of *A. dracunculus pilosa*, the majority substances, common to both phenophases were: eucalyptol, borneol, camphor and camfen. They were not identified in the composition of volatile oil of species *A. dracunculus*.

Therefore, we can say that the biosynthesis of volatile compounds included in the composition of oil extracted from species of *A. dracunculus* phenophase changes depending on species and variety.

Table 1

Composition of volatile oil extracted from *Artemisia dracunculus* species, harvested in different phenophases (% of total compounds)

| Name of substance | *Artemisia dracunculus* – Ecotype Bucharest | | *Artemisia dracunculus* – var. Pilosa Bucharest | |
|---|---|---|---|---|
| | Blooming | Excedeed Maturity | Blooming | Excedeed Maturity |
| Tri-cyclen | - | - | 0.42 | 0.19 |
| α-Thujona | 0.70 | - | 0.07 | 0.35 |
| α- Pinen | 0.51 | 0,19 | 0.90 | - |
| Camphen | - | - | 6.60 | 3.42 |
| Sabinene | 51,06 | 32.26 | 0.45 | 0.16 |
| β- Pinene | - | 0.17 | 0.75 | 0.31 |
| Octen-3-ol | - | - | 0.26 | 0.14 |
| Terpinolene | - | - | 0.15 | 0,18 |
| Mircene | 1.92 | 1.08 | - | - |
| α -Terpinene | 2.94 | 1.07 | 0.53 | - |
| Limonene | 0.92 | 0.26 | - | - |
| β -Cymene | - | - | - | 1.46 |
| Eucalyptol | - | - | 44.46 | 38.25 |
| trans-β-Ocymene | 1.86 | 0.87 | - | - |
| o-Cymene | - | 0.28 | - | - |
| cis-β–Ocymene | 7.41 | - | - | - |
| γ–Terpinene | 4.25 | 1.72 | 0.83 | 0.34 |
| Ocymene | - | 4.40 | - | - |
| Izopropyl methyl ciclohexan-1-ol | 0.41 | 0.34 | 0.25 | - |
| Terpinolene | 1.96 | 1.01 | 0.20 | - |
| Izopropyl methyl bicyclo hexan 2-ol | - | 0.27 | - | - |
| cis-β Terpineol | 0.40 | - | - | - |
| Camphor | - | - | 9.93 | 5.61 |
| Izobornyl alcohol | - | - | 0.16 | - |
| Pinocarvona | | | 0.19 | |
| Borneol | - | - | 28.29 | 23.31 |
| Terpinen 4-ol | 7.21 | 4.10 | 2.35 | 1.53 |
| α –Terpineol | - | 0.15 | 0.80 | 0.48 |
| p-Cymene 8-ol | - | - | 0.13 | - |
| Estragol | 0.63 | 0.26 | - | - |
| Mirtenol | 0.51 | - | - | 0.34 |

| | | | | |
|---|---|---|---|---|
| Verbenona | - | - | 0.25 | - |
| Bornil format | - | 0.69 | 0.56 | 0.64 |
| Carvona | - | - | 0.08 | - |
| Piperitona | - | - | 0.11 | - |
| Bornil acetate | - | - | 0.37 | 0.39 |
| Cetronellyl acetate | 1.39 | 3.04 | - | - |
| Neryl acetate | - | 0.71 | - | - |
| Methyl eugenole | 7.1 | 3.53 | - | - |
| Geranyl acetate | 0.53 | - | - | - |
| Methyl isoeugenol | 0.24 | 0.78 | - | - |
| Elixen | - | 0.22 | - | - |
| β -Selinen | - | 0.31 | - | - |
| Elemicina | 3.06 | 6.24 | - | - |
| Neroledol | - | 0.21 | - | - |
| β -Cadinen | 0.24 | 0.35 | - | - |
| Isoelemicina | - | 33.76 | - | - |
| α- Cadinol | 0.66 | - | - | - |
| Azarone | 4.77 | - | - | - |

## CONCLUSIONS

The foliar blade of *Artemisia dracunculus var. pilosa* species presents secretory multicellular hairs and in the *Artemisia dracunculus* species there is a single secretory channel.

The transition from flowering stage to exceeded maturity determines the decrease of sabinene, terpinene-4-ol, methyl eugenol, gamma terpinene content and the disappearance of cis-beta-ocimene.

Eucalyptol, borneol, camphor and carmine are chemical compounds considered to be major and specific only to *Artemisia dracunculus var. pilosa.*

The biosynthesis process of volatile compounds included in the composition of oil extracted from *Artemisia dracunculus* species is influenced by phenophase and diversity of species.

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# RESULTS REGARDING THE INFLUENCE OF CLIMATIC CONDITIONS UPON THE DYNAMICS OF THE EVOLUTION OF "KEY" PATHOGENS IN CASE OF GRAPEVINE, VINEYARD OREVIȚA MARE BETWEEN 2010 AND 2011

Balaban Mihaela[1], Mitrea Rodi[2]

***Keywords****: grapevine, variety, behaviour, pathogen, attack.*

***Abstract:*** *The knowledge of the evolution of pathogen attacks in case of grapevine according to a specific year, together with the knowledge of the behaviour of the grape variety characteristic to the study area represent the key factor of the scientific approach of prevention and control of natural attacks specific to each pathogen.*

## INTRODUCTION

During the vegetation period, the grapevine is susceptible to the attack of certain damaging agents, among which we mention the pathogens: *Plasmopara viticola*, *Uncinula necator*, and *Botryotinia fuckeliana* that are present within all vineyards, at all varieties of *Vitis vinifera* (Mitrea R., Tudosie R., 2011).

Under favourable environmental conditions, the attack of the three aforementioned pathogens can trigger significant economic loss, which means phytosanitary protection represent the most important technological link due to direct influence upon both the production quality and the quantity (Irimia N., 2011).

In order to succeed, control programs have to rely on the knowledge of the behaviour of different varieties that are subject to pathogen attacks, while treatments should rely on the concomitant utilization of the three criteria – biological, ecological and phenological (Cebanu V. și colab., 2007). Under this context, the establishment of the interaction among the biology of the host, the ecology of the pathogen agent and the climatic elements represents the first step in identifying the most efficient prevention and control measures.

In Romania, the behaviour of different grapevine varieties to the attack of the three types of fungi in various crop producing regions was studied by numerous researchers (Georgescu & Dragu 1979, Heintz et. al. 1985, Blaich & Stein 1986, Eliade, 1990, Iacob 2000, Mitrea 2000, Dobrotă 2007, 2008) knowing the fact that the same variety may behave differently from one region to another mainly due to environmental factors.

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## MATERIAL AND METHOD

The studied biological material was represented by four grapevine varieties (Sauvignon Blanc, Cabernet Sauvignon, Tămâioasă românească – Romanian Tămâioasă and Fetească neagră – Black Fetească) cultivated at Orevița Mare farm, which belongs to Vânju Mare-Orevița vineyard.

The results regarding the dynamics of the evolution of the pathogens *Plasmopara viticola*, *Uncinula necator* and *Botryotinia fuckeliana* were registered in the climatic conditions of the years 2010 and 2011 (Table 1).

The estimation of the attack generated by the three pathogen agents was performed according to the methodology used in the Forecast and Warning Stations. For each pathogen agent and each variety, there was established the frequency (F%), intensity (I%) and it was calculated the degree of attack (DA%), the gathered data being processed according to some usual formula. In order to render the behaviour of the grapevine varieties cultivated on the vineyard Orevița Mare to the studied pathogens it was used an evaluation scale (Table 2) proposed by O.I.V. 1983 (Code $N^{OS}$ OIV 452, 453,455,456,458).

The evaluation of the studied varieties behaviour was made according to the method: VR = very resistant (without attack or mark 9); R= resistant (DA%= 1-10 or marks 7-8); MR= mean resistant (DA%= 10-25 or marks 5-6); S= sensitive (DA%= 25-50 or marks 3-4); VS= very sensitive (DA>50 or marks 1-2).

## RESULTS AND DISCUSSION

Besides temperature, precipitation amounts and relative humidity are factors that directly influence the dynamics of the pathogen agents' evolution. Thus, by analysing the evolution of the main pathogens that produce damages to the grapevine **from Oravița Mare farm**, under the climatic conditions characteristic to the years 2010-2011, we have noticed:

In case of the pathogen *Plasmopara viticola*, the **Forecast and Warning Station Drobeta Turnu Severin** indicated the application of certain preventive treatments according to each year, knowing that the fungus resists as oospores and the primary infection may occur when the soil surface is wet for 2-3 days and displays a temperature higher than 10-11$^{o}$C and the leaves have a 5 cm diameter; secondary infections may occur repeatedly during the vegetation period at minimum temperatures of 12-13$^{o}$C, in the presence of water drops on the susceptible organs (leaves and berries).In 2010, a very difficult year from the climatic viewpoint, the pathogen had optimal conditions for generating the infections on May 3, May 14, June 2, June 7, July 6, July 13, August 4 and August 15, thus being recommended 8 treatments.Under the climatic conditions of 2011, in order to prevent the attack of the pathogen *Plasmopara viticola*, there were applied 6 treatments on May 2, May 17, May 30, July 1, July 18 and August 3.By analysing the data regarding the evaluation of the behaviour of grapevine varieties cultivated in Orevița Mare vineyard to the attack of the pathogen *Plasmopara viticola* (Table 3), we may notice that, in the two research years, the 4 varieties behaved differently, being classified as varieties with mean resistance (Tămâioasă românească and Fetească neagră), character expression 5 and as resistant varieties (Sauvignon Blanc and Cabernet Sauvignon), character expression 7.

Climatic elements during the grapevine vegetation period that influence the pathogen attack, farm Vânju Mare-Oravița, 2011-2012

Table 1

| Year | April | | | May | | | June | | | July | | | August | | | September | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T °C | P l/m$^2$ | U % | T °C | P l/m$^2$ | U % | T °C | P l/m$^2$ | U % | T °C | P l/m$^2$ | U % | T °C | P l/m$^2$ | U % | T °C | P l/m$^2$ | U % |
| 2010 | 11.9 | 47.8 | 67.6 | 17.2 | 103.8 | 67.6 | 20.5 | 157.4 | 68.6 | 23.4 | 47 | 67.3 | 24.1 | 29.2 | 63 | 17.7 | 23.0 | 67 |
| 2011 | 11.8 | 1.8 | 63.13 | 16.45 | 4.5 | 71.5 | 21.4 | 5.6 | 62.9 | 22.8 | 3.98 | 67.4 | 23.5 | 4.4 | 57.3 | 22.2 | 2.8 | 52.4 |

Scale for evaluating the resistance of grapevine to the main pathogen agents

Table 2

| **Evaluation of resistance on leaves** | Character expression | Degree of attack (DA%) |
|---|---|---|
| Very weak | 1 | >75% |
| | 2 | 50-75% |
| Weak (large necrotic spots on the leaves) | 3 | 40-50% |
| | 4 | 25-40% |
| Mean (isolated necrotic spots of about 1 cm in diameter) | 5 | 15-25% |
| | 6 | 10-15% |
| Good (very small necrotic spots) | 7 | 5-10% |
| | 8 | 1-5% |
| Very good (without any symptoms of attack) | 9 | <1% |
| **Evaluation of the resistance on grapes** | 1 | >75% |
| | 2 | 50-75% |
| Very weak | 3 | 40-50% |
| | 4 | 25-40% |
| Weak (all grapes are attacked, they fall down) | 5 | 15-25% |
| | 6 | 10-15% |
| Mean (up to 20% of the grapes are shrivelled or rotted) | 7 | 5-10% |
| | 8 | 1-5% |
| Good (only few grapes are shrivelled or rotted) | 9 | <1% |
| Very good (without any symptoms of attack) | 1 | >75% |
| | 2 | 50-75% |

Evaluation of the behaviour of certain grapevine varieties to the attack of the pathogen *Plasmopara viticola* on leaves in 2010-2011

Table 3

| Variety | Mean DA% 2010-2011 | Character expression | Variety resistance |
|---|---|---|---|
| Fetească neagră | 18.3 | 5 | MR |
| Tămâioasă românească | 20.7 | 5 | MR |
| Sauvignon Blanc | 9.5 | 7 | R |
| Cabernet Sauvignon | 6.7 | 7 | R |

In case of the pathogen *Uncinula necator*, depending on the biological reserve acquired during the previous year and on the climatic conditions registered during the period of vegetative repose, it may be established the forecast of the pathogen evolution for the next year, as the main wintering form of the parasite is that of resistant mycelium in the bark and especially in the infested buds.

The pathogen attack is favoured by higher temperatures, the optimum around 22-26$^{o}$C, and thus, it is more frequent in dry summers. After the occurrence of the infections, the evolution of the disease is favoured by temperatures of 18-25$^{o}$C and a relative humidity of 50-80%. The fungus spores (conidia) do not germinate in water drops and abundant precipitations prevent the evolution of the disease.

In 2010, the Forecast and Warning Station Drobeta Turnu Severin recommended the application of a preventive treatment on May 5.

The attack was noticed on May 11 and the generalization occurred during the first decade of July at a mean temperature of 21.7$^{o}$C and a mean relative humidity of 71%.

Under the climatic conditions of 2011, similarly as in 2010, the first treatment was recommended by the beginning of May, the generalization of the attack being forecasted for July, when environmental conditions reached the optimum. As we may notice from Table 4, all the 4 grapevine varieties registered mean values of the degree of attack (DA%) that varied between 2.5% and 25.8%, depending on the variety.

A good behaviour was registered by the varieties Sauvignon Blanc and Cabernet Sauvignon (character expression 7, respectively 8).

Evaluation of the behaviour of certain grapevine varieties to the attack of the pathogen *Uncinula necator* on leaves in 2010-2011

Table 4

| Variety | Mean DA% 2010-2011 | Character expression | Variety resistance |
|---|---|---|---|
| Fetească neagră | 25.8 | 4 | S |
| Tămâioasă românească | 19.9 | 5 | MR |
| Sauvignon Blanc | 6.1 | 7 | R |
| Cabernet Sauvignon | 2.5 | 8 | R |

As in case of all pathogen agents, besides temperature, precipitations represent a limitative or favouring factor for the development of the pathogen *Botryotinia fuckeliana*. At the same time, relative humidity plays a decisive role in triggering the infections generated by the pathogen, as well as in re-starting the attack after long periods of drought.

For this pathogen, the optimum development temperature varies between 22 and 24°C; when there are also registered abundant rainfalls and water drops maintain on the grapes for 12-15 hours, the damages are huge.

In 2010-2011, for the fungus *Botryotinia fuckeliana*, the first treatment was recommended in the first decade of July; the potential state of infection reappeared in the second decade of this month.

Between the mellowness and ripening stages, the risk of infection decreased very much due to the reduced precipitation amounts registered from the second decade of August until the end of September.

Due to the environmental conditions registered during the research period that were less favourable to the attack of the fungus *Botryotinia fuckeliana* in the vineyard Orevița Mare, the cultivated varieties presented lower values of the degree of attack, between 0.8% and 2.6%, and thus, they were classified as resistant varieties with the character expression 8 (Table 5).

Evaluation of the behaviour of certain grapevine varieties to the attack of the pathogen *Botryotinia fuckeliana* on grape berries in 2010-2011

Table 5

| Variety | Mean DA% 2010-2011 | Character expression | Variety resistance |
|---|---|---|---|
| Fetească neagră | 1.3 | 8 | R |
| Tămâioasă românească | 2.6 | 8 | R |
| Sauvignon Blanc | 1.9 | 8 | R |
| Cabernet Sauvignon | 0.8 | 8 | R |

## CONCLUSIONS

In the period 2010-2011, in the vineyard Orevița Mare, it was signalled the simultaneous attack of the pathogens *Plasmopara viticola*, *Uncinula necator* and *Botryotinia fuckeliana*.

During the research period, for each pathogen agent the Forecast and Warning Station Drobeta Turnu Severin, recommended different treatments depending on the ecology of the pathogen, the phenological phase of the grapevine and the evolution of the climatic conditions.

In case of the pathogen *Plasmopara viticola*, the attack on the leaves registered a mean value varying between 6.7 and 20.7%, in case of *Uncinula necator*, the value of the mean degree of attack was between 2.5 and 25.8%, while for *Botryotinia fuckeliana*, the mean value of the degree of attack on grape berries oscillated between 0.8 and 2.6%.

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# INTERSPECIFIC RELATIONS CHARACTERISTIC TO OREVIȚA – MARE VINEYARDS, MEHEDINȚI COUNTY

Balaban Mihaela[1], Mitrea Rodi[2]

***Keywords:*** *producer, consumer, relations, agro-ecosystem, vineyard*

***Abstract****: Implementation of the ecological laws at the level of vineyard ecosystems contained, between the prevailing elements and phytosanitary protection sequences which have the chance to impose themselves by balanced solutions and by knowing the interdependent relations between the producers and consumers, at the vineyard biocenosis level.*

## INTRODUCTION

In the vineyard ecosystems take place interactions between: primary periodical abiotic factors, secondary periodic (Climate factors), periodical or nonperiodic (Physical factors), nonperiodic abiotic and biotic factors (Edaphic factors) and secondary periodic biotic (Intraspecific and interspecific relations) within the vineyard biotype, biocenosis and autocenosis.

As Olteanu & Mărăcineanu 2007 mention, the mutual influence between the vineyard and the environment is different, and during time it suffered profound changes by the intervention of new factors, as: pathogen agents, animal pests, new varieties, which determined the change of the initial established culture system. Within the new culture systems, the protection of the vineyards played and has to play an important role, being able to guarantee the profitability of the culture. This way, it is enhanced the natural vocation of the vineyard ecosystem at the level of the four subsystems (vineyard biocenosis, vineyard biotype, agrophytotechnical component and the social and economic subsystem (Leț & Rotea 1997). To succeed it is necessary to consider that the vineyard biocenosis is a semi artificial bioenosis, constituted from the vineyard plantation and all its organisms which live in trophic correlations and of other nature, stable (Stan et al., 2008). The aspects regarding the biodiversity from some vineyard agroecosystems presents Mitrea et al. 2008, Mitrea et al. 2009, coming to understand the need to apply specific measures of fighting the damaging agents.

## MATERIAL AND METHOD

The foundation of interspecific relations from within the Orevița Mare vineyard plantation is based on a plurality of data collected from the field. This way there were identified the grass varieties and there was established the spectrum of pathogens agents and the animal pests, due to the application of preventive measures with the role to maintain the attack degree and the wed status at a level as low as possible.

The natural status of weed was appreciated by the counting the grass species on meter square and their framing on two groups: annual or perennial monocotyledonous and dicotyledonous.

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The spectrum of active pathogens during the vegetation period was determined by direct observations on the field and laboratory determinations with the aid of the binocular loupe and of the ML4 microscope. To establish the entomofauna there were performed collections from the entomological material in various stages of vegetation of the vineyard, using different means and procedures: surveys in the soil and on the surface of the soil, collections with the entomological net, pheromone traps, food traps, visual checks, field analysis in the field or in the laboratory with the aid of the binocular loupe and the microscope to detect the mite vines. The collected species were analyzed and identified according to the determinations from the beams published in "Romania's Fauna".

## RESULTS AND DISCUSSIONS

In the Orevița Mare vineyard plantation from the territory of Mehedinți County, the species complex and the interspecific relations among these have a characteristic configuration which we present in tables 1, 2, 3 and 4.

The primary producer is represented by 4 species of vines which are cultivated on different surfaces by a larger or smaller number of years (table 1).

Table 1

The species of vines cultivated on Orevița Mare plantation

| Variety | Surface (hectare) | Year of foundation of the culture. |
|---|---|---|
| Sauvignon Blanc | 35 | 1977 |
| Cabernet Sauvignon | 8 | 2008 |
| Tămâioasă românească | 7,30 | 2007 |
| Fetească neagră | 7,70 | 2008 |

Next to the vine, in all vineyard biocenosis there are also other primary producers, represented by different weed species. These can exercise an unfavorable influence without control on their demography, being accepted the fact that the vine and the weeds are mutually competing when their number reached a level which became critical.

In the studied plantation, the coverage degree of the soil with weeds is high, especially in the cultivated lot with Sauvignon Blanc variety thing explained also by the age of the culture.

The floristic composition of the weeds present in the Orevița Mare vineyard plantation (table 2) is represented by the monocotyledonous, which have 58,58% from the total number of species identified and also dicotyledonous, which have a percentage of 41,42%. The most well known represented species is *Agropyron repens* (couch grass), followed by *Crepis setosa* (plant yolk) and *Achillea sp* . Among the consumers, in the vineyard biocenosis, the first order is the best represented, the phytopathogen and animal pests being part of them. These can have as main host the vineyard which represents the main cause of production loss, and as secondary host the weed species which, this way, can serve as bridge in the resumption of the evolutionary cycles. Regarding the phytopatogene agents, in table 3 we present identified species, the reporting period, as well as their classification according to the gravity of attack.We consider that the establishing of the pathogene structure from each vineyard ecosystem presents a major importance, due to the changes which appear permanently in the vineyard culture technology and even of some periodic changes concerning precipitations and temperatures regime, some species change their mode of nutrition, going from potentially harmful species group into the pathogens main group . For

Regarding the interspecific relations with the primary producers it should be considered that, unlike other culture plants, the main pathogene agents of the vineyard don't have as intermediate hosts species of weed (herbaceous plants). This way, by performing control works (mechanical or hand hoeing, herbicides), it does not interrupt the evolutionary cycle of pathogens, but excessive weeds maintain a moist environment, predisposing the vines to pathogens attack. Regarding the Entomofauna from the Oreviţa - Mare vineyard, it was divided into species with economic importance, occasional species, potentially harmful species and migratory species, in order to establish interspecific relationships with primary producers (table 4).Regarding the nutrition, only a few *Eryophyes vitis* Page attack vines or different species of the genre *Vitis* (*Phylloxera vastatrix* Planch) , the remaining species are polyphagous. Polyphagous species identified can attack plants belonging to species of wild flora, but some of them are feeding themselves, preferably with vine ( *Anomala solida* Eric*., Lobesia botrana* Den et Schiff., *Sparganotis pilleriana* Den et Schiff*., Clysia ambiguella* Hb.) .Analyzing this aspect , is noted that the number of harmful species, whose evolutionary cycle is organically linked to the vine is limited, while the number of polyphagous species is relatively high. Consequently, it can be said that the herbaceous species are host plants for secondary species, migratory species and also potentially harmful and support for oviposition and development of new generations of pests and shelter against harsh environmental conditions as well as various attacks against predators.

Moreover, it is not neglected that weeds from the vineyards provide shelter and conditions for oviposition and development of new generations of predators of insect pest species of vines.

These data are highlighting that, to establish measures and means of prevention and pest control agents, should be considered also the interdependent relationship between producers and consumers specific to any vineyard ecosystem.

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**Table 2**

**The floristic composition from the Orevița - Mare vineyard**

| | MONOCOTYLEDONOUS | | | | | | DICOTYLEDONOUS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ANNUALS | Average number of | Average % of participation | PERENNIAL | Average number of | Average % of participation | ANNUALS | Average number of weeds/meter | Average % of participation | PERENNIAL | Average number of | Average % of participation |
| | *Dasypyrum (Haynaldia villosum)* | 10 | 4,92 | *Agropyron repens* (couch grass) | 98 | 48,21 | *Erigeron annuus* (bunghişor) | 6 | 2,95 | *Convolvulus arvensis* (bindweed) | 2 | 0,98 |
| | *Bromus japonicus*(obsiga) | 6 | 2,95 | *Lolium perenn*e (roi gras) | 5 | 2,46 | *Crepis setosa* (plant yolk) | 13 | 6,39 | *Epilobium sp.* (fluff) | 4 | 1,96 |
| | | | | | | | *Portulaca oleracea* (grasse plant) | 8,4 | 4,13 | *Cardaria draba (*cheese-cow plant) | 6,(6) | 3,24 |
| | | | | | | | *Potentilla argentea* (scrântitoarea) | 2 | 0,98 | *Sonchus arvensis* (susai) | 5 | 2,46 |
| | | | | | | | *Conyza canadensis* (bătrâniş) | 1 | 0,49 | *Artemisia absinthium* (mugwort) | 1 | 0,49 |
| | | | | | | | *Stellaria media* (chickweed) | 2 | 0,98 | *Lepidium draba* | 1 | 0,49 |
| | | | | | | | *Amaranthus retroflexus* (amaranth) | 1 | 0,49 | *Taraxacum officinale* (dandelion) | 2 | 0,98 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *Solanum nigrum* (beam) | 2 | 0,98 | *Achillea sp.* | 10 | 4,92 |
| | | | | | | | *Capsella bursa-pastoris* (Shepherd's Purse) | 5 | 2,46 | *Leontodon sp.* | 1 | 0,49 |
| | | | | | | | *Filago vulgaris* | 1,5 | 0,73 | *Rubus candicans* (Stubble blackberry) | 2 | 0,98 |
| | | | | | | | *Erigeron annuus ssp strigosus* | 4,5 | 2,21 | *Onopordon acanthium*(thorn , velcro of the donkey) | 1 | 0,49 |
| | | | | | | | *Xanthium italicum* (cornaci) | 2,25 | 1,10 | | | |
| TOTAL | 2 | 16 | 7,87 | 2 | 103 | 50,67 | 12 | 48,65 | 23,89 | 11 | 35,6 | 17,48 |

**Table 3**

**The spectrum of pathogens identified in the Orevița - Mare grape plantation**

| Nr. crt. | Pathogen | Name of the disease | Attacks reporting period | Classification of species |
|---|---|---|---|---|
| | VIRUSES | VIRAL | | |
| 1 | Grapevine fan leaf virus | Short - Nodar | January - December | unidentified |
| 2 | Grapevine vein mosaic | Nerve mosaic | January - December | unidentified |
| | BACTERIA | BACTERIOSIS | | |
| 1 | *Agrobacterium radiobacter – pv. tumefaciens* | bacterial cancer | January - December | secondary |
| | FUNGUS | MYCOSIS | | |
| 1 | *Plasmopara viticola* | Blight | May - October | principal |
| 2 | *Uncinula necator(Schw.) Burr.* | Mildew | April -October | principal |
| 3 | *Botryotinia fuckeliana – (de Bary) Whetzel* | Gray mold | January – October | principal |
| 4 | *Elsinoë ampelina (de Bary) Schear* | Antracnosis | April -August | secondary |
| 5 | *Pseudopeziza tracheiphilla – Mü Thurgan* | Red spot disease | April- June September-October | potentially harmful |
| 6 | *Septoria ampelina* | Septoria | May - October | potentially harmful |
| 7 | *Eutipa lata (Pers)Tul* | Eutiposi | January - December | unidentified |
| 8 | *Phomopsis viticola Sacc.* | Vine escoriosis | April - September | unidentified |

**Table 4**

**The entomofauna structure of Orevița - Mare vine plantation**

| Nr.crt. | Species with economic importance | Occasional species | Potentially harmful species | Migratory species |
|---|---|---|---|---|
| 1 | *Eryophyes vitis* Page | *Anomala solida* Er. | *Phylloxera vastatrix* Planch, Galicola form | *Amphimalon solstitiales* |
| 2 | *Tetranychus urticae* Koch. | *Melolontha melolontha* L. | *Vespa* spp. L. | *Rhyzotrogaraequinoctiales* Herb. |
| 3 | *Lobesia botrana* Den et Schif | *Polyphylla fullo* F. | *Otiorrhynchus ligustici* L. | *Hyphantria cunea* Drury |
| 4 | *Sparganotis pilleriana* Den. et Schif. | *Pulvinaria vitis* Targ. | *Agriotes spp.* L. | |
| 5 | *Clysia ambiguella* Hb. | | *Byctiscus betulae* L. | |

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**Vol. XVII ( LIII ) - 2012**

# EXPERIMENTAL RESEARCHES WATCHING THE NITROGEN COMPOUNDS REDUCTION FROM THE PHREATIC WATERS

Belingher Mihaela Liliana[1], Chimerel M. E[2]

***Keywords***: *pilot station ZW-10, ammonium, azotite, azotate*

## ABSTACT

*The study of processes for drinkable phreatic waters polluted with nitrites, nitrates and ammonium ions, performed with the pilot station ZW -10 sampled from digged shaft (wells having 6-12 m depth) it was performed based on three samples of water taken from individual households located in Gorj district. It have been chosen three locations due to results registered after performed analyzes, being the most representative in the content of nitrogen compounds. In this work are presented the results obtained to one of the three experiments.*

## INTRODUCTION

The nitrogen retention problem and of its compounds to source and also from waste waters, constitutes on of the current issues of the present technologies for waste water treatment, technologies that must carry out more efficient treatment conditions.

The water for the human consumption must be sanogenic, to be free of microorganisms, without parasites or substances which by number or by concentration not to constitute a potential health hazard for consumers, must meet the minimum requirements and to respect in the sampling points, the curent legal regulations (Pîslăraşu s.a., 1981) (Robescu s.a., 2004) (Trofin, 1983).

To achieve this goal it is necessary to improve the efficiency of the biological treatment of the waters sampled in order to make drinkable and to clean the waste waters discharged into emissary, as well as measures to reduce pollution at source.

## MATERIALS AND METHODS

The experimental researches for the treatment of groundwater polluted with nitrogen compounds were made with the pilot station ZW-10, shown in figure no.1, consisting of: Chemical Pretreatment Unit (PREC), Biological Pretreatment Unit (PREB), Ultrafiltration Unit ZW-10 (UF) (Mirel, 2009).

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[2] Romanian Waters National Administration, S.G.A.Gorj

The description of technological proces and of the reagents system:

The quantity of water subjected to the drinkable processes performed with the pilot station ZW-10 it was of 10 liters/sample. The duration of complete cycle of treatment was of 240 minutes (4 hours) and it is represented of three stages.

Figura no.1 - Pilot station ZW – 10

I Stage :

From the 10 liter container using a feed pump with diaphragm, the water was introduced in the Chemical Pretreatment Unit (PREC) respectively in polypropylene flocculator (PP) equipped with engine and mixer, where was treated with aluminium sulphate Al2(SO4)3 (1 g). The aluminium sulphate is mainly used as flocculant agent in drinking water purification. From the polypropylene flocculator, water reaches in the vertically contact bed from PP with conical bottom for water clarification. The necessary time for this stage was of 60 minutes representing 25% of the entire cycle.

II Stage :

The clarified water is passed using a hydrophore provided by PREC in Biological Pretreatment Unit (PREB), which consists of a PP tank, with 4 compartments, provided with adjustable walls in order to obtain aerobe and anoxic areas, necessary to carry out biological nitrification/denitrification processes for advanced polluted water sources treatment. PREB Unit is provided with feed pump for the aerobe compartments with an intake connection placed in anoxic area or in the processing tank.

The biological nitrification experiments were carried out in a trickling filter system with a fixed bed. The support material for trickling filter was Clinoptilolit volcanic tuff (source: Mârşad exploitation, processed at S.C.CEMACON S.A. Zalău ).

The aerobe area for nitrification processes consists of a PP tank equipped with an air blast system compressed, supplied from a compressor. This compressor was started taking place the tank aeration in aerobe area. The saturated water in oxygen (Cmed = 7 mg

O2/l) is pumped through trickling filter (PP tank serves as a trickling filter with volcanic tuff support). The trickling filter sowing with volcanic tuff support was performed without using a previously preparated inocul. The biomass development occurred more slowly through groundwater recirculation in system. The contact time was of 90 minutes being performed daily, for 30 days, corrections of ammonium ions in order to ensure an average value of 4 mg NH4-/l during the whole period of sowing. The phosphate concentrations of 0, 9-1, 2 mg/l and the bicarbonate of 300 mg/l from water allowed to provide the carbon and phosphorous source for the nitrifying microorganism growth without additional admixture.

From the aerobe area the water passes into anoxic area (anaerobe) for carrying out the processes of denitrification. The anoxic area consists of a PP tank (V = 30 l; L x l x H = 200x500x500 mm) equipped with two mixers (0 - 2000 rot/min; 8/1 W mixer) and with actuating engines which are started for the water mixing.

The necessary time for nitrification/denitrification processes was of 132 minutes representing 55% of the entire cycle.

III Stage :

From PREB water is passed on Ultrafiltration Unit ZW-10 (UE) which is provided with a process tank (Bioreactor) from PP containing membrane module vertically installed, pH-meter and the level transducer, Nitto Kohki blower which produces the air flow of low pressure for biological oxidation and for membrane cleaning, the water tank with inverse impulse from PP, the process pump Verder type fitted with frequency regulator, peristaltic drain pump Pro Minet type used for the bioreactor draining and for removing the excess sludge, peristaltic pump Pro Minet type for MC-1 acid charging used to perform cleaning operations of the membrane module, and for pH adjusting, plastic container for solution MC-1 (V = 5 l), peristaltic pump Pro Minet type, for sodium hypochlorite dosage used for cleaning operations of membrane module, sodium hypochlorite container, measurement and control instruments ( level sensor, pressure and vacuum indicator, pH-meter, thermometer) chemical reagents (MC-1 acid solution, phosphoric acid, sodium hypochlorite solution). The treatment process permanently maintained a neutral pH around value of 7. At the end of the treatment cycle, water was disinfected with sodium hypochlorite solution. The necessary time for UF was of 48 minutes representing 20% of the treatment process.

## RESULTS AND DISCUSSIONS

The water sampled from the digged pit from Scoarţa, village Cerat (Belingher, Chimerel, 2011), had the following initial and final parameters after treatment, presented in table no.1.

Table 1

Initial and final parameters after treatment

| Nitrogen compounds | Before treatment (mg/l) | After treatment (mg/l) | C.M.A. (mg/l) |
|---|---|---|---|
| Ammonium ($NH_4^+$) | 5 | 0,1 | 0,5 |
| Azotite ($NO_2^-$) | 1,45 | 0,05 | 0,5 |
| Azotate ($NO_3^-$) | 55 | 1,4 | 50 |

Graphical representation of the values obtained at ammonium indicator is given in figure no. 2.

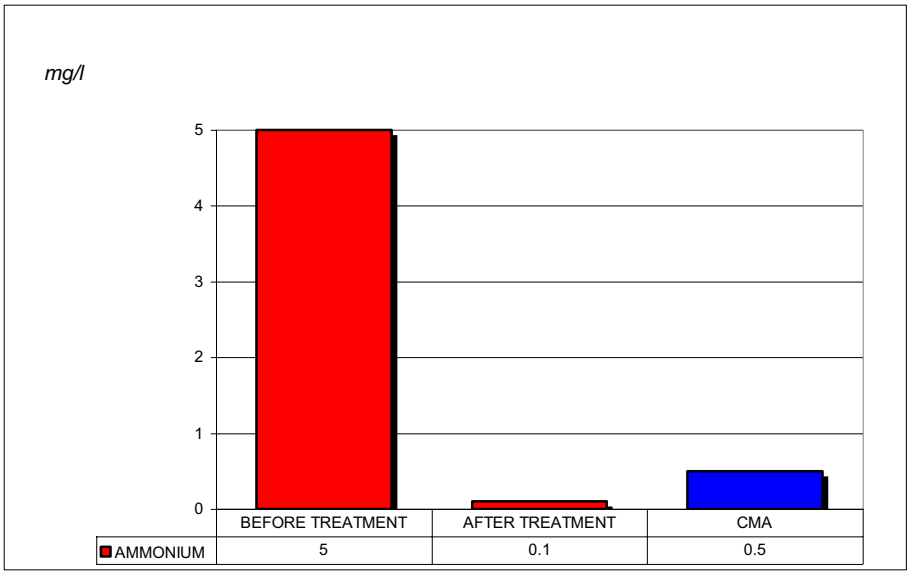


Figure no.2 Ammonium - Values obtained at ammonium indicator

Graphical representation of the values obtained at azotite indicator is given in figure no. 3.

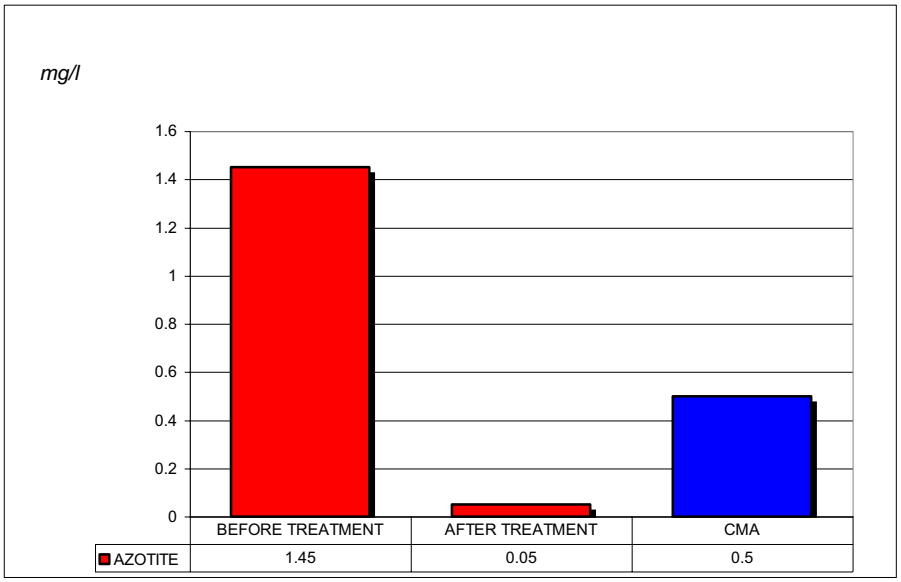


Figure no.3 Azotite - Values obstained at azotite indicator

Graphical representation of the values obtained at azotate indicator is given in figure no. 4.

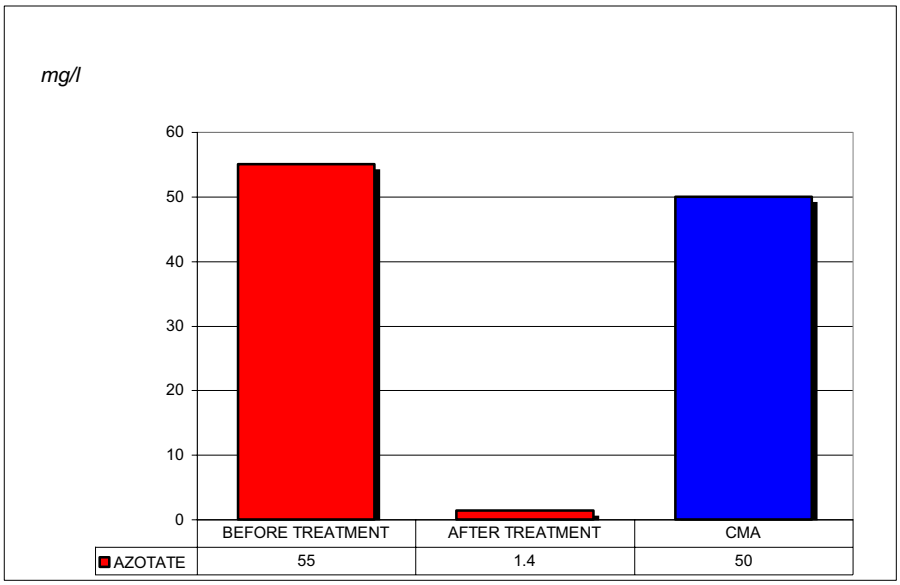


Figure no.4 Azotate - Values obstained at azotate indicator

The treatment efficiency accounting achieved with ZW-10 pilot station was made with the following formula:

$$E\% = \frac{Cinp - Cout}{Cinp} x100$$

Where: E% - Plant efficiency
$C_{inp}$ – Input concentration
$C_{out}$ – Outcome concentration

The ZW-10 pilot station efficiency in case of ammonium indicator:

$$E\% = \frac{5 - 0,1}{5} x100 = 98\%$$

The ZW-10 pilot station efficiency in case of azotite indicator:

$$E\% = \frac{1,45 - 0,05}{1,45} x100 = 96,56\%$$

The ZW-10 pilot station efficiency in case of azotate indicator:

$$E\% = \frac{55-1,4}{55} x100 = 97,45\%$$

The overall efficiency of ZW-10 pilot station for reducing/eliminating nitrogen compounds in case of sample I is:

$$E\% = \frac{98+96,56+97,45}{3} = 97,34\%$$

## CONCLUSIONS

In the chosen treatment method were used chemical, biological and ultra filtration processes, the obtained results being very good, and the overall treatment efficiency of the plant exceeding 97%. Taking into account the obtained results this method can be applied on large scale in waters treatment process necessary to subject to drinkable.

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# THE IPG GLOBAL POLLUTION INDEXES METHOD APLICATTION FOR THE GLOBAL THEORETICAL ASSESSEMENT OF THE IMPACT PRODUCED BY THE NITROGEN COMPOUNDS UPON PHREATIC WATERS IN GORJ COUNTY

Belingher Mihaela Liliana[1], Chimerel M. E[2]

**KEY WORDS**: *Global pollution index method, ammonium, phreatic water*

## ABSTRACT

*In order to asses the impact of the phreatic waters pollution by nitrogen compounds as well as for the time evolution of the pollution phenomen, we need a global evolution of these pollution degree at some time.*

*A proposed variant in these meaning is the global pollution index method.*

## INTRODUCTION

The phreatic water is the groundwater which is found on the first impermeable layer of the ground surface, and which supplies wells, springs, etc, influencing the formation and the properties of soil. The proper groundwater is under this impermeable layer.

The pollution with nitrates represents the environmental factors loading, waters (surface and groundwater) and soil with nitrogen compounds, whose consequences are: endangering the human health, damaging the living organisms and the terrestrial and aquatic ecosystems, disturbing the water uses and damaging the natural ambience.

The chemical parameters of drinking water portability allowed by the current legislation are: 50 mg/l for nitrate ($NO_3$); 0,5mg/l for nitrites($NO_2$); 0,5 for ammonium ($NH_4$) (Law no.458/2002).

## MATERIALS AND METHODS

The localities from Gorj district in which were found high concentrations that exceed authorized limits of nitrogen are: Cerat-Scoarța and Bengeşti-Ciocadia, and to nitrogen were exceedings in wells from localities Săuleşti, Curpeni-Stăneşti, Bumbeşti-Jiu, Polovragi, Băleşti, Tămăşeşti, Curtişoara, Urdari, Turcineşti and Hurezani (figure no.1) (Belingher, Chimerel, 2011).

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[2] Romanian Waters National Administration, S.G.A.Gorj

Generally, it is considered that it is possible to asses the pollution degree from an area and at one time, by giving a note of creditworthiness for each quality indicator.

The creditworthiness note obtained for each quality indicator in the analyzed area, serve to graphical analyze of a diagram, as a simulation method of the synergistic effect produced by pollutants.

The ideal state is represented by a regular geometrical figure in which the circumradius that passes through the polygon peaks, is divided into ten units of creditworthiness, the count starting from the center of the circle.

By joining the resulted points from the values arrangement expressing the real state, is obtained a irregular geometrical figure with a little matte surface inscribed into a regular geometrical figure of the ideal state.

The index of global pollution of an ecosystem (IPG) result of the ratio between the surface, representing the ideal state (Si) and the surface representing the real state (Sr).

When there are no changes of the environmental quality factors, so when there is no pollution, this index is equal to 1(Lazăr, Dumitrescu, 2006).

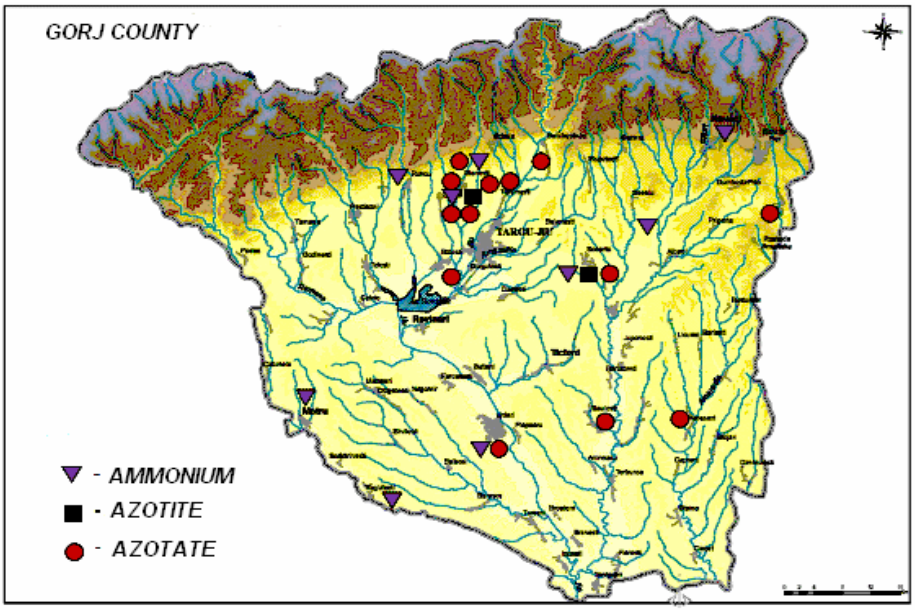


Figure 1-The map with the wells which were registered values above maximum permissible limits

Conventionally it was established a scale from 1 to 6 for the global pollution index.

Table 1

Scale from 1 to 6 for the global pollution index

| IPG VALUES | Effect upon the environment |
|---|---|
| 1 | The natural unaffected environment by human activity |
| 1÷2 | The environment submitted to the effect of the human activity within allowable limits |
| 2÷3 | The environment submitted to the effect of the human activity, causing |

| | |
|---|---|
| | the discomfort state of the life forms |
| 3÷4 | The environment submitted to the effect of the human activity, causing disturbances of the life forms |
| 4÷6 | The environment seriously affected by the human activity, dangerous for the life forms |
| > 6 | Degraded environment, inappropriate for the life forms. |

For the theoretical global assessment of the impact produced by the nitrogen compounds on the phreatic waters, it was appealed to the graphical simulation method of the synergistic effect done based on the creditworthiness note given for each pollutant included in the study (figure no.2).

The IPG global pollution indexes method adjusts for the three nitrogen compounds that constitutes in pollutants of phreatic waters.

Under these conditions the polygon becomes a triangle in whose peaks we pass the real values obtained as an average values for year 2011.

It was determined from analyzes the following average values:

- ammonium = 1,3 mg/l
- azotite = 0,12 mg/l
- azotate = 28, 43 mg/l

From the table with the creditworthiness notes resulted the following:

$N_{b\ ammonium} = 7$

The category of water on which frames according to the creditworthiness scale is the II category.

For nitrites and nitrates the values not exceeding the maximum permissible concentration, the creditworthiness note is 9. Note 10 is given if these pollutants were not present (respectively 0 mg/l).

$N_{b\ azotite} = 9$
$N_{b\ azotate} = 9$

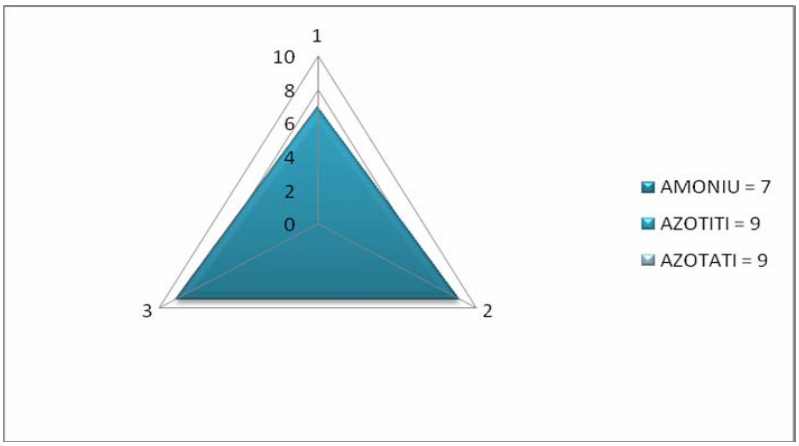


Figure 2. - I.P.G. for wells

## RESULTS AND DISCUSSIONS

$$IPG_{wells} = \frac{S_{ideal}}{S_{real}} = 1{,}5$$

Where: -$S_{ideal}$ = Ideal surface
-$S_{real}$ = Real surface

$$S_{ideal} = \frac{5{,}2x4{,}4}{2} = 11{,}44$$

$$S_{real} = \frac{4{,}5x3{,}4}{2} = 7{,}65$$

For IPG wells = 1,5 results that the phreatic waters are affected by the pollutants taken into study in alowable limits with the mention that it was worked with average values on the wells where it were found flagrant exceedings of CMA but also below this limit.

## CONCLUSIONS

For the groundwater catchwork in these areas, it is imperative necessary to draw up hydrogeological studies and hidrochemical ones, showing the real situations of the area, with special reference the nitrogen compounds thus the designers to provide or not treatment water plants for drinking water.

Prohibition of using drinking water from wells that have high concentrations of nitrogen compounds.

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***Law no.458/2002

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# MECHANISMS OF DROUGHT PERSISTENCE IN ROMANIA IN THE CLIMATE CHANGE PERSPECTIVE

Bojariu R., [1] Velea L.F.[1], Cică R. D. [1], Dobrinescu A. E. [1], Bîrsan M. [1], Dumitrescu A. [1]

## ABSTRACT

*We used Palmer index of soil moisture anomalies computed on monthly basis for 113 stations which cover Romanian territory to analyze the interdecadal variability in the time interval 1961-2010. The EOF analysis of Palmer soil moisture index shows a dominant interdecadal signal covering the whole Romanian territory which seems to be related to large scale phenomena. A second signal could be associated to climate change and it shows a tendency for depletion of soil moisture in southern and eastern regions of Romania.*

***Keywords*:** *Palmer index; soil moisture depletion; NAO; climate change signal.*

## INTRODUCTION

Drought has different definitions depending on the type of impact or socio-economic activity which is affected. From the meteorological point of view, a drought period is defined by a significant deficit in the precipitation regime. Pedological drought refers to a significant deficit in the soil moisture and has a significant impact for agricultural activities. In the context of climate change, the frequency and intensity of droughts are changing and their social and economical impact increase. The main goal of the present study is to analyze interdecadal variability and change of droughts in Romania in order to further identify their predictive potential associated to this time scale.

## DATA AND METHODOLOGY

The Palmer Drought Severity Index (PDSI) (Palmer, 1965) has been computed using the components of the local hydrological budget, taken into account the demand (e.g. evapotranspiration, runoff) and the supply of water resources (e.g. precipitation, water holding capacity of the soil) for a certain area (Palmer, 1965; Alley, 1984). Best results using PDSI are obtained for regions with relatively smooth topography, characterized by homogeneous physical and geographical conditions (Barbu and Popa, 2002).

In order to calculate PDSI for a certain month (i), one have firstly to determine the index of soil moisture anomaly Zi for that month (i):

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$$Z_i = k * \left(P - \alpha * PE - \beta * PR - \gamma * PRO + \delta * PL\right)$$

PDSIi= PDSIi-1 + 1/3*Zi - 0,103* PDSIi-1

where: k is an empirical weighting factor, specific for each region; α, β, γ, δ are coefficients for evapotranspiration, soil water recharge, run-off and water loss from the soil computed as weights of real quantities from potential ones, for each variable; P, PE, PR, PRO, PL represents observed precipitation, potential evapotranspiration computed using Thornthwaite method (Thornthwaite, 1984), potential recharge, potential run-off and potential water loss from the soil. Here we used the method and software developed by Wells at al. (2004) to compute self calibrated PDSI values and related indices. The self calibrated PDSI numerically match the behavior of the index at any location by replacing empirical constants of Palmer (1965) with newly calculated constants based on local climate.

In this study, we used only monthly values of Palmer index of soil moisture anomaly (Zi) which were computed for 113 stations covering the Romanian territory. Prior to computing PDSI, input data was homogenized and missing data was replaced, using software package M.A.S.H. (Szentimrey, 1998; Szentimrey, 2007; Szentimrey, 2011; Lakatos et al, 2011). The period of interest here is 1961-2010. Soil moisture measurements from agro-meteorological platforms relevant for maize and wheat indicated large values, statistically significant, of their correlation coefficient with the Palmer index Zi. Reference data used to test the representativeness of Zi index for the soil moisture anomalies in Romania covered the Southern part of Oltenia (Bojariu et al, 2012). The analysis of changes and variability of the monthly filtered values of the Palmer index for the soil moisture anomalies has been performed using the method of Empirical Ortogonal Functions (EOF) decomposition. Data used in the EOF analysis was first filtered using a 12-months running mean.

## RESULTS

In order to focus the analysis on the interdecadal component of the variability, initial data was low pass-filtered using a 12-months running mean.

The EOF analysis of the Palmer index for the monthly soil moisture anomalies computed for the 113 stations available in Romania indicates the existence of an interdecadal variability covering the entire Romanian territory, yet more pronounced in the southern regions (figure 1). The variance associated to this mode represents 50% of the total variance of the data field.

The second EOF mode of the monthly soil moisture anomalies indicates a tendency towards soil moisture depletion in the southern and eastern parts of Romania. The depletion signal is stronger in the Baragan region (figure 2). At the same time, there is another signal associated with a tendency of increasing soil moisture in the north-western part of the country (figure 2). The variance associated with this mode represents 10% of the total variance of the data field.

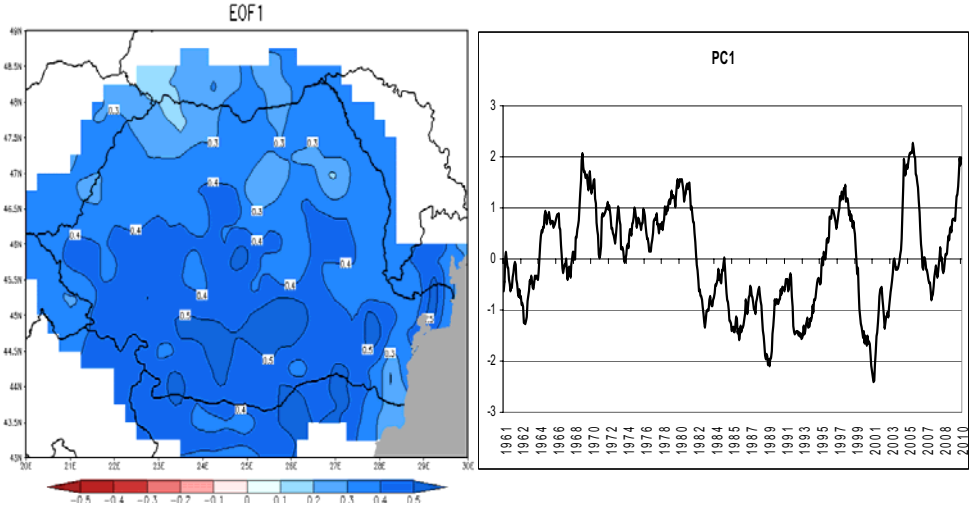


Figure 1. First EOF (spatial pattern and associated time series) of the Palmer index for the monthly soil moisture anomalies computed for the 113 station in Romania (1961-2010). Initial data was filtered using a 12-months running mean. The variance associated to this mode is of 50% of the total variance of the data field.

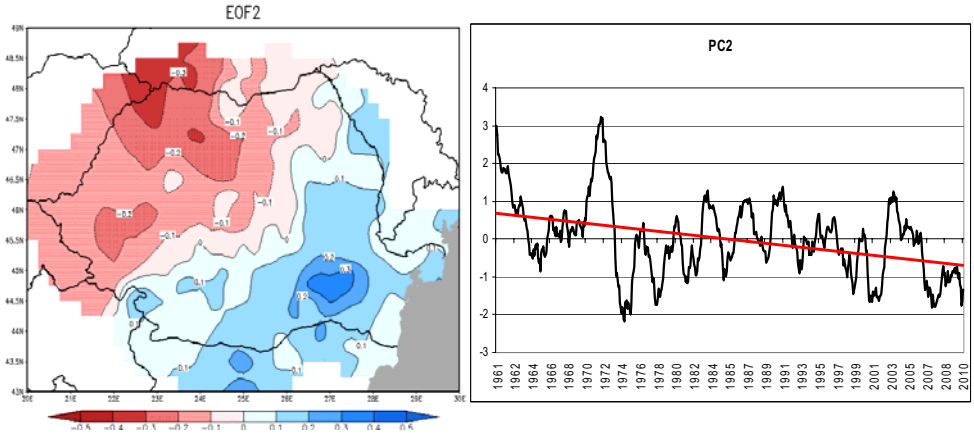


Figure 2. Second EOF (spatial pattern and associated time series) of the Palmer index for the monthly soil moisture anomalies computed for 113 stations in Romania (1961-2010). Initial data was filtered using a 12-months running mean. The variance associated to this mode is of 10% of the total variance of the data field.

## CONCLUSIONS

The spatial pattern of the first EOF mode (same sign over the entire Romanian territory) suggests the existence of a large-scale generating mechanism which acts on interdecadal time scale. This mechanism seems to be related to the natural variability of the climate system, the variation tendency being less clear (figure 1). The second EOF mode seems to be related to the climate change signal. It suggests for the Romanian territory, at annual time scales, a tendency for aridization especially in the southern part of the country, due to changes in the fields of precipitation, temperature and evapotranspiration during the warm season. Furthermore, the projections of the future climate obtained with global and regional climate models suggest the same pattern of the changes which is similar to the second EOF mode, for the hydrological regime in Romania (figure 3).

Among the candidate phenomena responsible for the generation of the signal associated to the first EOF of soil moisture anomalies there are Atlantic Multidecadal Oscillation (AMO) and the North-Atlantic Oscillation (NAO). Relationships between these phenomena and the interdecadal variability of the soil moisture anomalies in Romania seem to be non-linear as correlation coefficients are not very high between their associated indices. These aspects related to the climate mechanisms responsible for the soil moisture variability will be clarified in a forthcoming study.

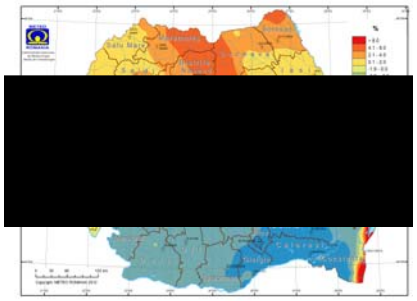

Figure 3. Changes of the multi-annual means of the evapotranspiration (in %) for 2011-2040 compared with the reference period 1961-1990. The means of an ensemble of 9 future projections using 9 regional climate models (FP6 ENSEMBLE) have been used.


## ACKNOWLEDGEMENTS

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**Vol. XVII ( LIII ) - 2012**

# MORPHO-ANATOMICAL STUDY ON THE RHIZOME FROM THE *ALCHEMILLA XANTHOCHLORA* SPECIES

Boruz Violeta[1]

***Keywords****: Alchemilla xanthochlora*, *rhizome*, *anatomy*, *Romania*

## ABSTRACT

*This paper presents the morpho-anatomical features of the rhizome from the Alchemilla xanthochlora Rothm. species. The material investigated from anatomical point of view was collected from Parâng Mountains (Groapa Seacă, located at an altitude of 1598 m). There were realized cross sections through the rhizome, analyzing its structure. Anatomical descriptions accompanied by micrometer measurements and original photographs.*

## INTRODUCTION

The *Alchemilla* species are mostly mountainous, subalpine and alpine plants – they are perennial, small to medium sized. Their identification is usually difficult to make. For a correct recognition of the species, one needs, beside a rich specialty literature, a material to compare and a rich collection of samples of mature plants.

For complete descriptions of *Alchemilla* species and to highlight any structural differences between these species identified in Parâng Mountain Groups were made histo-anatomical studies on vegetative organs.

The anatomy of vegetative organs to *Alchemilla* species is less approached in the consulted literature, on the national and international level. Missing data with reference to anatomy vegetative organs at *A. xanthochlora*.

In this paper there are analyzed morpho-anatomical aspects of the rhizome from the *Alchemilla xanthochlora* species, collected from Parâng Mountains (Romania). It is a medium-sized plant to very large, 15-90 cm tall, fairly rigid, often yellowish-green, with rhizome of 5-15 mm thickness.

## MATERIAL AND METHODS

For identification of the *Alchemilla xanthochlora* species there been used the specialty literature (Buia 1956, Ciocârlan 2009, Fröhner 1990, Walters & Pawłowski 1968).

The material of *Alchemilla xanthochlora* investigated from anatomical point of view, represented by rhizome, comes from Parâng Mountains. The plants have been preserved in grain alcohol 70 %, to the full flowering of the plants.

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The sections and microscopic preparations were taken into account the methodology of Tarnavschi et al. 1974.

Microscopic preparations were made by sectioning with the hand microtome. There were realized transversal sections through the middle section of the rhizome and flowering stem. Clarification and coloring sections were made according to the methodology of Andrei & Paraschivoiu 2003.

Microscopic examination of preparations was made to Nikon microscope and the photographs were made using a Nikon camera. Measurements were made on MC-3 microscope. There were about 10 measurements (epidermal cells, the wood vessels diameter). There have been taken into consideration the maximum individual value ($V_M$), the minimum individual value (Vm) and average ( $\overline{x}$ ) which is the arithmetic average of the 10 individual values.

## RESULTS AND DISCUSSIONS

Rhizome (Fig. 1a)

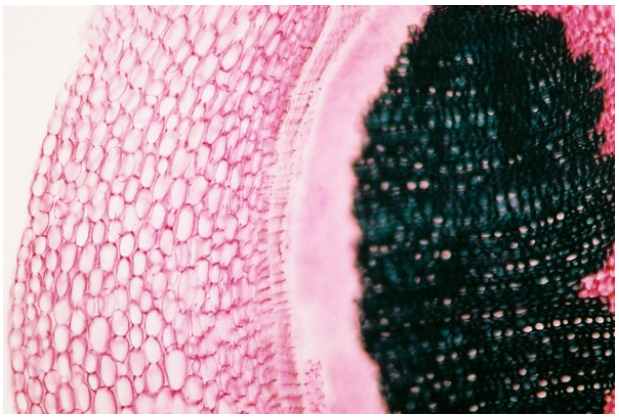

Fig. 1a. Cross section at *A. xanthochlora* rhizome: the epidermis, hypodermic tabular collenchym, the bark itself, the endodermis, the suber, the secondary phloem, the secondary xylem (Oc. 10x, Obj. 10, Orig.)

The secondary structure of this perennial underground axis is more evident at the central cylinder.

*The epidermis* is discontinuous in many places by exfoliation due to the pressure exerted by secondary internal tissues. Where it maintains, the cells maintain large, tangentially elongated, with external walls little thickened, covered with a cuticle of 1.8 μm. The epidermis has thickness of 20.25 μm.

*The bark* is pluri-layered (14-15 layers), composed of tangentially elongated cells. The first layer under the epidermis is easy collenchymatous. The other layers are composed of polygonal-rounded cells with intercellular, unequal spaces. The average thickness is 360 μm.

*The endodermis* is discontinuous and when is evident, is composed of small cells, tangentially elongated with slightly thickened walls.

*The suber* (Fig. 1b) formed by felogen activity which differentiated in the pericycle is pluri-layered (12-14 layers of cells) with known characteristics. It has a thickness of 112.5 μm. From place to place the suber is interrupted by large collenchymatous cells.

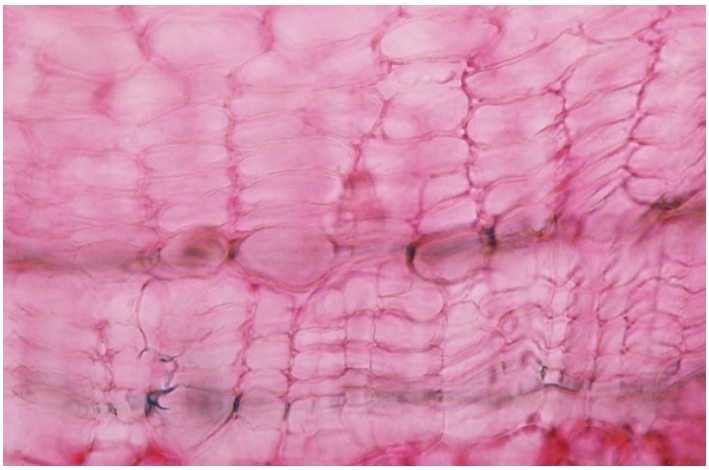

Fig. 1b. Cross section at *A. xanthochlora* rhizome: Structural detail at the suber pluri-layered, interrupted from place to place by large collenchymatous cells (Oc. 10x, Obj. 40, Orig.)

*The secondary conducting tissues* (Fig. 1c): the phloem and xylem are highligted clearly in section.

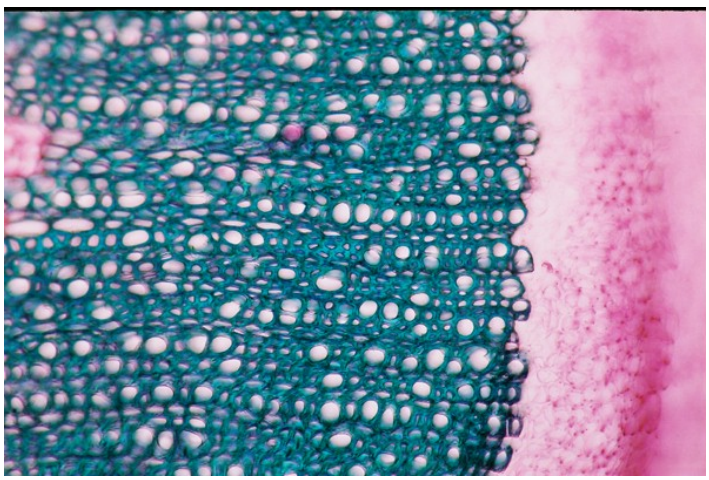

Fig. 1c. Cross section at *A. xanthochlora* rhizome: structural detail at the conducting secondary tissues: the phloem secondary as a thin ring, the secondary xylem much thicker and medullary rays (Oc. 10x, Obj. 20, Orig.)

The secondary phloem has thickness of 108 μm and consists of sieve tubes, annexed cells and more phloem parenchyma. It looks like a thin ring on outside.

The secondary xylem is much thicker (238.5 μm), consisting of xylem narrow vessels, polygonal, arranged in radial rows. Tere is a lot of wooden lignified parenchyma and is not observed libriform. It highlights the medullary rays uni- and pluriseriate. The diameter of the xylem vessels decreases centrifugal, towards the phloem: $V_M$ = 15.75 μm; Vm = 6.75 μm; $\overline{x}$ = 10.575 μm. There are not observed distinctly elements of the primary xylem.

*The medullary parenchyma* is well developed, composed of polygonal rounded cells, with pecto-cellulose walls and intercellular spaces.

The first histo-anatomical investigations on a species of *Alchemilla* were made in Romania by Toma & Rugină 1998, and refers to *Alchemilla vulgaris* L. species. *A. vulgaris* is also presented in the work of synthesis "Anatomy of the Dicotyledons" (Metcalfe & Chalk 1950), but here there can be found only a few references on the structure of this species.

## CONCLUSIONS

The rhizome shows a secondary structure in the central cylinder. Medullary parenchyma is well developed, there have not been observed calcium oxalate crystals.

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**Vol. XVII ( LIII ) - 2012**

# THE LEAF MORPHO-ANATOMY OF *ALCHEMILLA XANTHOCHLORA* SPECIES

Boruz Violeta[1]

***Keywords***: *Alchemilla xanthochlora, leaf, anatomy, Romania*

## ABSTRACT

*In this paper are presented the morpho-anatomical peculiarities of the leaf on Alchemilla xanthochlora Rothm. species (Rosaceae family). There have been performed transversal sections (through the petiole in the middle section and through the lamina of the median nerve) and tangential sections through the foliar limb (excoriations of the epidermis), analyzing the adaxial and the abaxial epidermis, in order to highlight the stomata, their form and dimensions, the epidermal cells and the hairs. The descriptions of anatomical features of the leaf are accompanied by micrometer measurements and original photographs.*

## INTRODUCTION

Knowledge morpho-anatomical features of *Alchemilla* species is a necessary objective dictated by the difficulties posed by this genre on the taxonomic level. The anatomy of vegetative organs to *Alchemilla* species is less approached in the consulted literature, on the national and international level. Missing data with reference to anatomy vegetative organs at *A. xanthochlora*.

In this context, the paper presents the morpho-anatomical aspects of the leaf at *A. xanthochlora* species.

Plant of a medium up to high size, up to 15-90 cm, often yellowish-green. The basal leaves (Fig. 1) with long petiols, reniform to orbicular lamina, little funnel shapped to plane, rarely waved, thin, yellowish-green for a long time.

Adaxially glabrous leaves or with few hairs on teeth, seldom along the folds or along all the surface of the lamina with few hairs, abaxially more or less densely hairy, seldom with hairs only on the side and nervures, divided up to 1/4-1/3 in 7-11 semiorbicular lobes, with acute, subequal teeth.

Petiols of 1.5-3 mm thickness, adaxially flat, soft and densely hairy, with erect-patent or patent hairs.

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Fig. 1. *A. xanthochlora* – basal leaf detail (orig.)

## MATERIAL AND METHODS

For identification of the *A. xanthochlora* species there have been used the specialty literature (Buia 1956, Ciocârlan 2009, Fröhner 1990, Walters & Pawłowski 1968).

The material of *A. xanthochlora* investigated from anatomical point of view, represented by basal leaves, comes from the Parâng Mountains (Groapa Seacă). It has been preserved in grain alcohol 70 % to the full flowering of the plants.

The sections and microscopic preparations were taken into account the methodology of Tarnavschi et al. 1974.

Microscopic preparations were made by cuttings with the hand microtome. There were realized transversal sections from the basal leaf (through the petiole in the middle section, and through the lamina in the median nerve level) and tangential sections through the foliar limb (excoriations of the epidermis), analyzing the adaxial and the abaxial epidermis, in order to highlight the stomata, their form and dimensions, the epidermal cells and the hairs.

Clarification and coloring sections were made according to the methodology of Andrei & Paraschivoiu 2003.

Microscopic examination of preparations was made to Nikon microscope and the photographs were made using a Nikon camera.

Measurements were made on MC-3 microscope. There were about 10 measurements (epidermal cells, the stomata, the hairs) or 10 counts (the stomata and the hairs).

There have been taken into consideration the maximum individual value ($V_M$), the minimum individual value (Vm) and average ( $\overline{x}$ ) which is the arithmetic average of the 10 individual values.

## RESULTS AND DISCUSSIONS

***A. xanthochlora* -** the leaf anatomy:

The petiole in transversal section (Fig. 2) has a semicircular contour and presents three steles (polistellical structure).

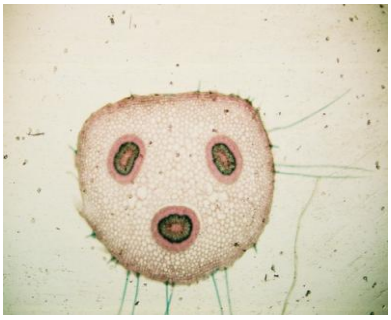

Fig. 2. Transversal section through the petiol of the *A*. *xanthochlora* (Oc. 10x, Obj. 6, Orig.)

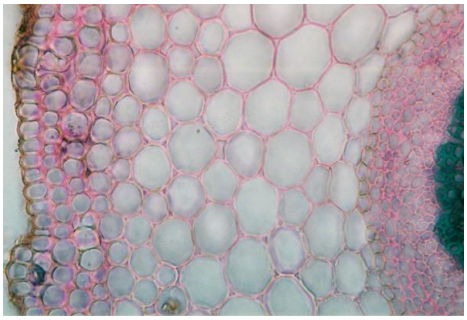

Fig. 3. Transversal section of the petiole at *A*. *xanthochlora*. Structural detail: the epidermis, tabular collenchyma, the crust, the endodermis, phloem, area of xylem (Oc. 10x, Obj. 40, Orig.)

The epidermis (Fig. 3) is unilayered, composed of isodiametric cells with relatively thin walls, the external curved and covered with a cuticle of 2.025 μm. The epidermis has a thickness of 15.75 μm. On the epidermis there can be observed unicellular simple hairs and with a sharp points.

The crust (Fig. 3) is a fundamental parenchyma. The first two layers are converted into tabular collenchyma. Between the three steles there can be observed emerging some air gaps.

The three conducting bundles that make up the three steles are arranged as follows: two towards the adaxial face, which is almost flat and the third, the largest, towards the convex abaxial epidermis.

All steles are surrounded by their own endodermis and have a known structure: the phloem and the xylem are arranged concentrically, and in the central area is the parenchyma (Fig. 4).

Fig. 4. Transversal section of the petiole at *A*. *xanthochlora*. Structural detail of the median stele at *A*. *xanthochlora*, with the phloem and the xylem in the form of concentric rings, in the center with medullary parenchyma (Oc. 10x, Obj. 20, Orig.)

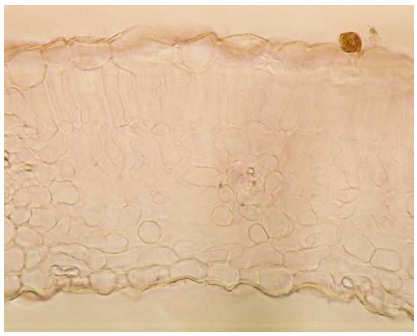

Fig. 5. Transversal section through the leaf blade in the *A*. *xanthochlora*: adaxial epidermis, palisade parenchyma bilayer; the lacunose parenchyma, the abaxial epidermis with stomata (Oc. 10x, Obj. 40, Orig.)

Diameter phloem vessels: $V_M$ = 11.25 μm; Vm = 4.5 μm; $\overline{x}$ = 7.875 μm.

The xylem consists mainly of xylem vessels that grow in diameter in centrifugal direction. The vessels are arranged in radial rows and accompanied by wood parenchyma.

Diameter xylem vessels: $V_M$ = 13.5 μm; Vm = 6.75 μm; $\overline{x}$ = 11.7 μm.

The leaf lamina – in transversal section:

Analyzing transversal section through the limb (Fig. 5) there can be seen that the leaf is bifacial type as in other species, with thickness of 180 μm.

The adaxial epidermis is unilayered, composed of elongated cells in tangential direction, covered with a cuticle of 1.575 μm thick. The average thickness of the epidermis is 22.5 μm.

The mesophyll contains palisadic and lacunose assimilation parenchyma and the nerve.

The palisade parenchyma is bilayer, the first layer with elongated cells perpendicular to the epidermis, and the second consisting of shorter cells with evident intercellular spaces. The thickness of the parenchyma: 90 μm.

The lacunose parenchyma is composed of polygonal cells with large intercellular spaces. It has a thickness of 70.4 μm, slightly narrower than the palisade.

The nerve (Fig. 6) represents the conducting phloem-xylematic fascicles, as an arch with adaxial opening. It seems surrounded by a parenchymatous sheath.

The abaxial epidermis is unilayered, with hairs and stomata. It is 15.75 μm thick and the cuticle of 1.575 μm.

Fig. 6. Transversal section trough the leaf blade in the *A*. *xanthochlora*. Median nerve (Oc. 10x, Obj. 40, Orig.)

Tangential section through the limb. The epidermis

a. Adaxial epidermis (Fig. 7) is composed of polygonal cells with irregular outline, with moderately wavy lateral walls, without intercellular spaces.

Length of epidermal cells: $V_M$ = 72 μm; Vm = 33.75 μm; $\overline{x}$ = 52.2 μm. The width of epidermal cells: $V_M$ = 33.75 μm; Vm = 22.5 μm; $\overline{x}$ = 27.9 μm.

As annexes of the epidermis are the stomata of anomocytic type, and the hairs very rare.

Length of the stomata cells: $V_M$ = 27 μm; Vm = 22.5 μm; $\overline{x}$ = 24.3 μm. Density of the stomata: $V_M$ = 149/mm$^2$; Vm = 64/mm$^2$; $\overline{x}$ = 98/mm$^2$.

The hairs are very rare and with a known structure.

b. *Abaxial epidermis* (Fig. 8) consists of polygonal cells, larger than those on the adaxial face, with wavy stronger lateral walls.

Length of epidermal cells: $V_M$ = 58.5 μm; Vm = 38.25 μm; $\overline{x}$ = 47.025 μm.

The width of epidermal cells: $V_M$ = 36 μm; Vm = 22.5 μm; $\overline{x}$ = 27 μm.

As annexes there are stomata and hairs.

Anomocitic type stomata are disposed ± regular. Length of the stomata cells: $V_M$ = 29.25 μm; Vm = 24.75 μm; $\overline{x}$ = 27.45 μm. Their density is higher than those on the adaxial epidermis: $V_M$ = 212/mm$^2$; Vm = 127/mm$^2$; $\overline{x}$ = 178/mm$^2$.

The hairs are unicellular, elongated, simple and pointed at the ends. Length of the hairs: $V_M$ = 918 μm; Vm = 558 μm; $\overline{x}$ = 712.8 μm. Density of the hairs: $V_M$ = 29/mm$^2$; Vm = 12/mm$^2$; $\overline{x}$ = 16/mm$^2$.

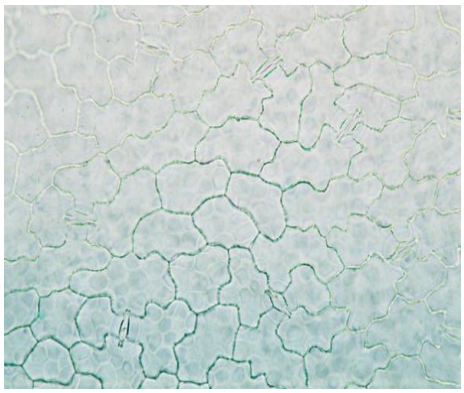

Fig. 7. Adaxial epidermis with few stomata in *A. xanthochlora* (Oc. 10x, Obj. 40, Orig.)

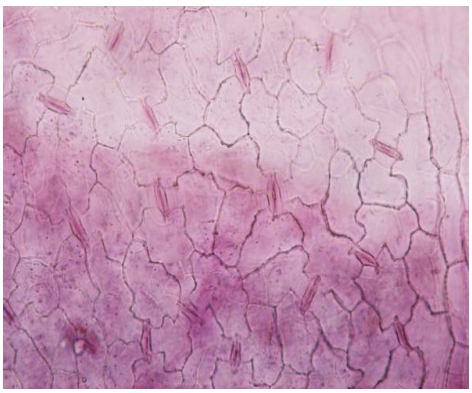

Fig. 8. Abaxial epidermis with larger epidermal cells and more numerous stomata and disposed more or less uniform in *A. xanthochlora* (Oc. 10x, Obj. 40, Orig.)

The leaf anatomy to *Alchemilla* is less approached in the consulted literature. The first histo-anatomical investigations on a species of *Alchemilla* were made in Romania by Toma & Rugină 1998, and refers to *Alchemilla vulgaris* L. species and the petiol structure is missing its description. *A. vulgaris* is also presented in the work of synthesis "Anatomy of the Dicotyledons" (Metcalfe & Chalk 1950), but here we find only a few references on the structure of this species.

## CONCLUSIONS

The petiole in transversal section has a semicircular contour and presents three steles (polistellical structure).

Analyzing the transversal section through lamina of the leaf, it is found that the leaf is bifacial type, as in other species of *Alchemilla*.

Analyzing the tangential section through lamina of the leaf it shows that the adaxial epidermis is composed of polygonal cells, with thin lateral walls, moderately wavy. The abaxial epidermis is composed of polygonal cells, larger than those on the adaxial side, with lateral walls wavier.

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✔*Horticultură*
✔*Tehnologia prelucrării produselor agricole*
✔*Ingineria mediului*

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# THE ENERGY VALUES OF HIGH-YIELD PLANTS GROWN IN "SHORT ROTATION COPPICE" BIOMASS SYSTEMS

Botu I.[1], Stoian I.[2], Vicol A.[1], Lazar A.[1] , Alecu A.[1]

***Key words***: *energy, dry biomass, caloric power, genotypes*

## ABSTRACT

*The energy value of woody plants (Salix, Populus, Paulownia) grown in Northern Oltenia in Short Rotation Coppice (S.R.C.) system is determined by biomass yield (41.7 to 71.1 t/ha) and dry heat power or 40% humidity (20.10/ 17.365 and 10.43/9.01 mg/kg).The levels of biomass/ha is similar in case of domestic selections and foreign varieties. Dry biomass production varied between 11.6 t/ha/year ('Cozia 3') and 16.8 t/ha/year ('Paulownia T1'). High calorific of dry biomass (0% humidity) varied from 17.36 to 20.1 MJ/kg and lower calorific from 16.92 to 18.40 MJ/kg. If the humidity level is 40%, net calorific value varies between 9.01 and 10.43 MJ/kg, returning to one ha of SRC system type between 172.3 x $10^3$ and 262.5 x $10^3$ MJ / ha.*

## INTRODUCTION

The demand for clean energy without a high level of pollution determined the use of woody plants for producing biomass yields.

At world level different species (willow, poplar, black walnut, *Paulownia*, etc.) are used in Short Rotation Coppice (SRC) system with short rotation cycle and annual vegetative regeneration. In this respect there was demand for obtaining new varieties with rapid growth and high biomass yields (willow cultivars in Sweden and England, poplar in Italy, etc.) (Lindegaard s.a., 2001, Karp si Shied, 2008). In Romania have been introduced sporadically some varieties of these plants and have been studied in terms of morphological behavior in different environmental conditions, without having to specify precisely how their behavior.These cultures provide multiple benefits including: providing a renewable energy, clean, high calorific value, low residue, 1-25/30 ratio of favorable energy consumption per energy produced, positive influence in preserving ecosystems and so on, are only some of which led to addressing this energy purposes issue.Research work started in 2009 at University of Craiova - SCDP Valcea focused on SRC type trials designed for establishing the most valuable introduced varieties along with the selections of natural hybrids of *Salix, Populus* and other genus. In addition with the evaluation of the performance of these genotypes in plantation, we intend to establish the energy value of biomass and according to this aspect to choose the most valuable genotypes that can be used later in commercial culture.

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## MATERIAL AND METHODS

The research activity was carried out from 2009 till 2012 at University of Craiova - SCDP Valcea in collaboration with SC CET Govora SA.

The biological material used consisted in 10 *Salix* genotypes, 3 of *Populu*s and one of *Paulownia tomentosa.* Out of the 14 genotypes studied, 7 are of domestic origin.

The SRC system trial was designed using randomized blocks method, with 4 repetition and 30 plants per plot, with double rows distanced at 2.0 x 0.7 x 0.6 m (12,328 plants/ha), except in case of 'Paulownia T1' where the planting distances for single rows were 2.0 x 0.8 m (6250 plants/ha).

Experimental data was collected from the trial during 3 years and was related to:

- amount of biomass per hectare at harvest phase (t/ha);
- amount of biomass at 40% moisture level (t/ha);
- amount and percentage of dry matter (t/ha/year);
- ash content of dry matter (% and t/ha);
- biomass calorific dry substance:
    - high calorific value (MJ/kg);
    - lower calorific value (MJ/kg);
    - net calorific value at 40% moisture biomass (MJ/kg MJ/ha).

During vegetation periods no irrigation was provided. The average rainfall level for the study period was 373.4 mm (April-July). Biomass harvesting was carried out during the dormant period (in December).

## RESULTS AND DISCUSSION

In terms of the environmental conditions from the University of Craiova - SCDP Valcea trial, the 14 genotypes studied behaved differently. Biomass yields were obtained in the 2nd, 3rd and 4th years after planting and varied in average from 41.7 t/ha ('Tordis') to 71.1 t/ha ('Paulownia T1').Generally, all genotypes reveal a increase biomass production starting from the second year (38.5 t/ha) till the fourth year (70.5 t/ha) (Table 1).

Under the conditions of Northern Oltenia the average yields (3 years) of the studied willow cultivars as 'Inger' (57.4 t/ha), 'Sven' (55.8 t/ha), and 'Cozia 1' (55.5 t/ha) proved significant positive yields of biomass, the poplar cultivar 'I 214' (61.3 t/ha) produced significant positive yield and 'Sacrau T1' (67.6 t/ha) and 'Paulownia T1' (71.1 t/ha) very significantly positive yields in comparison with the control cultivar Tordis' (41.7 t/ha), which is one of the valuable varieties in Europe. Even the domestic willow selections 'Pesred' (54.1 t/ha), 'Robisal' (49.6 t/ha), 'Cozia 3' (49.5 t/ha), 'Fragisal' (46.7 t/ha) and 'Dragasani 1' (50.9 t/ha) proved superior to control ('Tordis') but not statistically different according to analysis of variance. The results show up that in the given ecological conditions the studied willow genotypes are highly productive and of interest for producing clean energy.

Biomass production obtained at harvesting was brought up naturally to 40% moisture level (Table 2). In this case, the amount of biomass ranged from 16.68 t/ha ('Tordis') and up to 26.44 t/ha ('Paulownia T1'). The largest amounts were recorded at: 'Paulownia T1' (28.44 t/ha), 'Sacrau T1' (27.04 t/ha), 'I 214' (24.52 t/ha), 'Inger' (22.96 t/ha), 'Cozia 1' (22.20 t/ha), etc.

Table 1

Biomass yields in the first three years of production in SRC system using different willow, poplar and *Paulownia* cultivars and selections (2nd to 4th year after planting)

| Species | Cultivars/ Selections | Biomass yield at harvest (t/ha) | | | | Difference ± | Significance |
|---|---|---|---|---|---|---|---|
| | | 2nd year | 3rd year | 4th year | Average | | |
| *Salix* sp. | `Tordis` | 25.9 | 27.6 | 71.5 | 41.7 | - | - |
| *Salix* sp. | `Tora` | 32.3 | 42.4 | 61.4 | 45.4 | +3.7 | |
| *Salix* sp. | `Inger` | 39.4 | 63.8 | 69.0 | 57.4 | +15.7 | * |
| *Salix* sp. | `Jorr` | 345 | 37.5 | 65.3 | 45.8 | +4.1 | |
| *Salix* sp. | `Sven` | 36.9 | 57.7 | 72.7 | 55.8 | +14.1 | * |
| *Salix* sp. | `Cozia -1` | 40.6 | 55.5 | 70.3 | 55.5 | +13.8 | * |
| *Salix* sp. | `Pesceana 7' (`Pesred`) | 39.8 | 42.4 | 80.1 | 54.1 | +12.4 | |
| *Salix* sp. | `Robesti 1` (`Robisal`) | 39.4 | 35.5 | 73.9 | 49.6 | +7.9 | |
| *Salix* sp. | `Cozia casanta` (`Fragisal`) | 30.8 | 49.0 | 60.4 | 46.7 | +5.0 | |
| *Salix* sp. | `Cozia 3` | 36.9 | 46.3 | 65.4 | 49.5 | +7.8 | |
| *Populus* sp. | `I 214` | 51.4 | 64.1 | 68.4 | 61.3 | +19.6 | ** |
| *Populus* sp. | `Sacrau T1` | 40.7 | 88.0 | 74.2 | 67.6 | +25.9 | *** |
| *Populus* sp. | `Dragasani 1` | 33.8 | 50.4 | 68.4 | 50.9 | +9.2 | |
| *Paulownia* | `Paulownia T1` | 56.7 | 76.5 | 80.1 | 71.0 | +29.4 | *** |

LSD 5.0%= 13.87; LSD 1.0%=18.68; LSD 0.1%=24.84

Table 2

Biomass production in SRC plantations of different cultivars and selections depending on the moisture content

| Species | Cultivar/ Selection | Biomass production at harvest an average of 3 years (t/ha) | Production of biomass at 40% moisture (t/ha) | DM percentage of total biomass (%) | Production of dry biomass/ha/ year (average 3 years) | DM ash content | |
|---|---|---|---|---|---|---|---|
| | | | | | | % | t/ha |
| *Salix* sp. | `Tordis` | 41.7 | 16.68 | 29.0 | 12.1 | 0.79 | 0,096 |
| *Salix* sp. | `Tora` | 45.4 | 18.16 | 29.3 | 13.3 | 0.85 | 0,113 |
| *Salix* sp. | `Inger` | 57.4 | 22.96 | 22.3 | 12.8 | 0.76 | 0,097 |
| *Salix* sp. | `Jorr` | 45.8 | 18.32 | 27.3 | 12.5 | 0.73 | 0,091 |
| *Salix* sp. | `Sven` | 55.8 | 22.32 | 22.6 | 12.6 | 0.82 | 0,103 |
| *Salix* sp. | `Cozia -1` | 55.5 | 22.20 | 22.9 | 12.7 | 0.80 | 0,102 |
| *Salix* sp. | `Pesceana 7' (`Pesred`) | 54.1 | 21.64 | 21.8 | 11.8 | 0.78 | 0,092 |
| *Salix* sp. | `Robesti 1` (`Robisal`) | 49.6 | 19.84 | 25.6 | 12.7 | 0.77 | 0,098 |
| *Salix* sp. | `Cozia casanta` (`Fragisal`) | 46.7 | 18.68 | 25.5 | 11.9 | 0.74 | 0,088 |
| *Salix* sp. | `Cozia 3` | 49.5 | 19.80 | 23.4 | 11.6 | 0.73 | 0,085 |
| *Populus* sp. | `I 214` | 61.3 | 24.52 | 23.0 | 14.1 | 0.82 | 0,115 |
| *Populus* sp. | `Sacrau T1` | 67.6 | 27.04 | 21.3 | 14.4 | 0.71 | 0,102 |
| *Populus* sp. | `Dragasani 1` | 50.9 | 20.36 | 27.5 | 14.0 | 0.74 | 0,104 |
| *Paulownia* | `Paulownia T1` | 71.1 | 28.44 | 23.6 | 16.8 | 0.91 | 0,153 |

Determinations carried out on the level of dry biomass (0% humidity), revealed that the production of dry matter (DM) varied between 11.6 t/ha/year ('Cozia 3') and 16.8 t/ha/year ('Paulownia T1'). If the amount of DM at *Salix* genotypes was between 14.0 to 14.4 t/ha/year, in case of 'Paulownia T1' was 16.8 t/ha/year. These levels of DM per ha are close to those obtained in other countries, small differences are caused by local environment and soil conditions.DM percentages of biomass at harvest varied between 21.8% ('Pesred') to 29.3% ('Tora'). Biomass ash content ranges from 0.71% ('Sacrau T1') to 0.91% ('Paulownia T1'), returning a quantity of 0.085 t/ha ('Cozia 3') to 0.153 t/ha ('Paulownia T1'). The amount of residue level is very low compared to that resulting from

the burning of fossil fuels (coal). The difference between the amount of DM and ash is due to carbon, hydrogen, sulfur, nitrogen and other minerals.The determinations performed on calorific were related both to dry, with 0% humidity and the biomass with 40% moisture (Table 3). The dry high heat output is different from one genotype to another and oscillate between 17.36 MJ/kg ('Dragasani 1') and 20.1 MJ/kg ('Cozia 1').Lower calorific value ranged from 16.92 MJ/kg ('Dragasani 1') to 18.42 MJ/kg ('Cozia 1'). Differences between foreign varieties and local selections are very low both in terms of high and low calorific levels. If the biomass has a moisture content of 40% the calorific values vary from 9.01 MJ/kg ('Dragasani 1') to 10.43 MJ/kg ('Tora'), differences between genotypes being very small.At one hectare of SRC culture the 40% moisture biomass have achieved net calorific values from 172.3 x $10^3$ Mj/ha ('Tordis') to 262.5 x $10^3$ Mj/ha ('Paulownia T1'). Net calorific value of 200 x $10^3$ Mj/ha was achieved for 'Paulownia T1', 'Sacrau T1', 'I 214', 'Pesred', 'Cozia 1', 'Inger' and 'Sven', returning over 47.778 $10^3$ kcal/ha (1 Mj = 238.89 kcal) or 155.510$^3$ kWh/ha (1Mj = 0.7778 kWh) in International System of Units.These relative calorific values of the biomass are very important because can be obtained consistently per one hectare of SRC type culture for a period of 20-25 years with the condition of using of adequate soils and crop technologies.

Table 3

Calorific values of biomass resulted from different cultivars and selections in SRC plantation

| No. | Species | Cultivar/ Selection | Dry matter quantity of biomass (t/ha) | Dry calorific (0% humidity) | | Net calorific value at 40% moisture | |
|---|---|---|---|---|---|---|---|
| | | | | High calorific (Mj/Kg) | Low calorific (Mj/Kg) | Mj/Kg | Total production (Mj/Kg) |
| 1 | *Salix* sp. | `Tordis` | 16.68 | 19.10 | 17.70 | 10.73 | 172.3 x $10^3$ |
| 2 | *Salix* sp. | `Tora` | 18.16 | 19.78 | 18.40 | 10.43 | 188.8 x $10^3$ |
| 3 | *Salix* sp. | `Inger` | 22.96 | 18.95 | 18.15 | 10.21 | 234.4 x $10^3$ |
| 4 | *Salix* sp. | `Jorr` | 18.32 | 19.30 | 18.22 | 9.82 | 179.9 x $10^3$ |
| 5 | *Salix* sp. | `Sven` | 22.32 | 19.50 | 18.36 | 9.64 | 215.1 x $10^3$ |
| 6 | *Salix* sp. | `Cozia -1` | 22.20 | 20.10 | 18.42 | 9.88 | 219.3 x $10^3$ |
| 7 | *Salix* sp. | `Pesceana 7' (`Pesred`) | 21.64 | 19.16 | 18.25 | 9.91 | 214.4 x $10^3$ |
| 8 | *Salix* sp. | `Robesti 1` (`Robisal`) | 19.84 | 19.31 | 17.95 | 9.88 | 196.0 x $10^3$ |
| 9 | *Salix* sp. | `Cozia | 18.68 | 18.91 | 18.05 | 9.94 | 185.6 x |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | casanta` (`Fragisal`) | | | | | $10^3$ |
| 10 | *Salix* sp. | `Cozia 3` | 19.80 | 18.85 | 17.89 | 9.56 | 189.3 x $10^3$ |
| 11 | *Populus* sp. | `I 214` | 24.52 | 17.49 | 17.02 | 9.78 | 239.8 x $10^3$ |
| 12 | *Populus* sp. | `Sacrau T1` | 27.04 | 17.88 | 17.05 | 9.05 | 244.7 x $10^3$ |
| 13 | *Populus* sp. | `Dragasani 1` | 20.36 | 17.36 | 16.92 | 9.01 | 183.4 x $10^3$ |
| 14 | *Paulownia* | `Paulownia T1` | 28.44 | 19.06 | 17.76 | 9.23 | 262.5 x $10^3$ |

## CONCLUSIONS

The energetic value of woody plants (*Salix, Populus, Paulownia*) grown in the SRC system in Northern Oltenia was given by biomass yields (41.7 to 71.1 t/ha) and calorific values of dry and/or 40% humidity level biomass (20.10/17.36 to 10.43/9.01 MJ/kg). Higher biomass yields at harvest were obtained for: 'Paulownia T1' (71.1 t/ha), 'Sacrau T1' (67.6 t/ha), 'I 214' (61.3 t/ha), 'Inger' (57.4 t/ha), 'Cozia 1' (55.5 t/ha), etc. Biomass levels are similar to local selections and foreign varieties.Production of dry biomass varied between 11.6 t/ha/year ('Cozia 3') and 16.8 t/ha/year ('Paulownia T1'), which is lower in case of *Salix* genotypes (11.6 – 13.3 t/ha/year) compare with those of *Populus* ones (14.0 to 14.4 t/ha/year);Biomass-ash content is very low in all genotypes (0.73 to 0.91%), which represent 0.085 to 0.153 t/ha.High-calorific value of dry biomass (0% humidity) vary from 17.36 MJ/kg to 20.10 MJ/kg and lower calorific value from 16.92 to 18.40 MJ/kg.The biomass is used in kilns at 40% moisture content and in this case the net calorific value vary between 9.01 and 10.43 MJ/kg, which represent for one hectare of culture between 172.3 x $10^3$ Mj/ha to 262.5 x $10^3$ Mj/ha.

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✔*Ingineria mediului*

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# STUDY ON BIOMASS PRODUCTION AND QUALITY OF BIOENERGY WILLOWS CULTIVATED IN THE NORTH AREA OF OLTENIA

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***Key words***: *bioenergy crop, biomass, willow, SRC, production*

**ABSTRACT**

*Under the conditions of North Oltenia the Short Rotation Coppice (SRC) type bioenergy plantations of willow achieved biomass yields between 41.7 and 57.4 t /h per year, comparable to these obtained in other countries. The Romanian willow selections produced from 46.7 t / ha per year ('Fragisal') to 55.5 t / ha per year ('Cozia 1'), similar to the most valuable foreign varieties. The yield efficiency of the SRC plantations increase from second year to fourth year, from 35.5 t / ha per year to 69.4 t / ha per year. In case of 373 mm rainfall from April till July lateral shoots occur on 24.3% on same year shoots in case of the foreign varieties and on 64.8% in case of Romanian selections. The occurrence of lateral shoots have a negative influence on dry biomass yield. When applying irrigation to complete the water deficit in the soil the percentage of lateral shoots decrease significantly. The Romanian selections, in addition to superior biomass yields, proved more resistant than foreign ones to pests and diseases.*

## INTRODUCTION

The value of the energy potential of several bioenergy crops of Short Rotation Coppice (SRC) type is dependent on the annual biomass which they produce and its quality.

According to the European Directive 2001/77/EC, biomass shall mean the biodegradable fraction of products made in different kinds of plantations of renewable resources.

In ancient times willows have been used as wood for different purposes (as materials to produce different products, in medicine, etc.) and only after 1970 as a renewable energy source (Karp et al., 2011).

Willows are a group of plants made up of 350-500 wild species (Argus, 2007); most of them originated in Asia (275) and less in Europe (65).

*Salix* gender exhibits very large genotypic and phenotypic variability, but for the production of biomass of superior quantitative and qualitative levels, rigorous selection into the spontaneous flora and/or obtaining new genotypes through various methods of breeding are required.

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At University of Craiova - SCDP Valcea has stared a complex programme of producing biomass through growing several foreign willow cultivars and also local selections of *Salix* suitable for SRC cultures type.

The final aim of the programme is to select the most valuable genotypes, with high potential for biomass production and high quality for SRC cultures in the North area of Oltenia, in order to produce clean energy.

## MATERIAL AND METHODS

The study was carried out during the period 2009-2012 in the trials of the University of Craiova - SCDP Valcea. The biological material used consisted in introduced *Salix* cultivars ('Tordis', 'Tora', 'Inger', 'Jorr' and 'Sven') and Romanian genotypes ('Cozia 1'; 'Pesceana 7' named later 'Pesred'; 'Robeşti 1' named 'Robisal' and 'Cozia casantă' named 'Fragisal'). The Romanian genotypes were selected by the team from University of Craiova - SCDP Valcea from natural hybrids from the spontaneous flora of Oltenia.

The genotypes were planted in a non-irrigated SRC type trial, in double-rows spaced at 2.0 x 0.7 x 0.6 m (12,328 plants/ha) using randomized blocks method, with 4 repetition and 30 plants per plot.

Observations and determinations referred to: growth vigour of plants (number and length of annual shoots, the number and length of annual lateral shoots); quantity of biomass per plant and per ha in three consecutive years; the influence of climatic conditions (temperature and precipitation) on growth and biomass yield during the studied years.

The trial was maintained clean of weeds through disc and between plants with hoes. During vegetation period 5 *phytosanitary* treatments against diseases (*Melampsora salicis* – willow leaf rust) and pests (*Melasoma populi* – poplar leaf beetle and *Tuberolachnus salignus* – giant willow aphid) were applied.

In autumn, after leaves have fallen, the harvesting of annual growths was done manually, the willow stems were cut back at 1-2 cm above the ground, after that the stump was covered with 2-3 cm of soil.

## RESULTS AND DISCUSSION

Romanian or introduced *Salix* genotypes behaved differently in terms of the growth in the Northern area of Oltenia. Biomass quantity obtained and its quality is dependent on genotype and environmental conditions.

Plants growth was recorded in the 2$^{nd}$ to 4$^{th}$ year after planting, those years being also the years of harvesting biomass. The main plant growth characteristics refer to the number of annual growth and their average length (Table 1). The average number of annual shoots was lower in the second year after planting (7.8 pcs.) and increased to 14.3 pcs. in the third year and 23.0 pcs. in the fourth year. Average number of shoots / plant in all the three years varied between 11.1 ('Tora') to 20.7 ('Inger').

Foreign or introduced cultivars have achieved an average of 15.7 shoots/plant and the Romanian selections 14.2 shoots, the differences between the two groups of genotypes are low. The average length of annual growth ranged between 2.78 m ('Cozia casantă' - 'Fragisal') and 3.58 m ('Inger'). The group of foreign willow cultivars overall growths by 0.36 m (in average) higher than Romanian selections.

Table 1

Plant growth characteristics of the studied cultivars and selections of *Salix* in SRC plantations

| No. | Cultivars / Selections | Average number of annual shoots/plant | | | | Average annual growth length (m) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 2nd year | 3rd year | 4th year | Mean | 2nd year | 3rd year | 4th year | Mean |
| 1 | 'Tordis' | 6.4 | 8.6 | 21.0 | 12.0 | 2.94 | 3.35 | 3.05 | 3.11 |
| 2 | 'Tora' | 7.6 | 10.7 | 15.0 | 11.1 | 2.70 | 4.04 | 2.83 | 3.19 |
| 3 | 'Inger' | 8.5 | 26.3 | 27.2 | 20.7 | 2.60 | 4.76 | 3.40 | 3.58 |
| 4 | 'Jorr' | 5.8 | 17.4 | 26.4 | 16.5 | 2.87 | 4.05 | 3.00 | 3.30 |
| 5 | 'Sven' | 7.8 | 19.2 | 27.1 | 18.0 | 2.95 | 4.27 | 2.98 | 3.40 |
| ***Mean of introduced cvs.*** | | ***7.2*** | ***16.4*** | ***23.3*** | ***15.7*** | ***2.81*** | ***4.09*** | ***3.05*** | ***3.31*** |
| 6 | 'Cozia 1' | 8.8 | 12.0 | 20.7 | 13.8 | 1.75 | 4.25 | 3.37 | 3.12 |
| 7 | 'Pesceana 7'('Pesred') | 9.7 | 12.5 | 19.0 | 13.7 | 2.71 | 3.10 | 3.50 | 3.10 |
| 8 | 'Robeşti 1'('Robisal') | 8.5 | 10.3 | 24.4 | 14.4 | 1.97 | 3.40 | 3.00 | 2.79 |
| 9 | 'Cozia casantă' ('Fragisal') | 7.0 | 12.0 | 26.2 | 15.1 | 2.00 | 3.64 | 2.70 | 2.78 |
| ***Mean Romanian selections*** | | ***8.5*** | ***11.7*** | ***22.6*** | ***14.2*** | ***2.10*** | ***3.40*** | ***3.14*** | ***2.95*** |
| ***General Mean*** | | ***7.8*** | ***14.3*** | ***23.0*** | ***14.8*** | ***2.50*** | ***3.87*** | ***3.09*** | ***3.15*** |

Biomass production (annual growths) harvested in the second year of growth is differentiated between genotypes (Table 2). The average amount of biomass in the three years of harvest oscillated between 41.7 t/ha ('Tordis') and 57.4 t/ha ('Inger'). Overall biomass production is high, including that of Romanian selections (46.7 to 55.5 t/ha) without irrigation.

Table 2

Biomass yield of *Salix* cultivars and selections in the first three years of harvest (2nd to 4th year)

| No. | Cultivars / Selections | Biomass yield at harvest (t/ha) | | | |
|---|---|---|---|---|---|
| | | 2nd year 2010 | 3rd year 2011 | 4th year 2012 | Mean |
| 1 | 'Tordis' | 25.9 | 27.6 | 71.5 | 41.7 |
| 2 | 'Tora' | 32.3 | 42.4 | 61.4 | 45.4 |
| 3 | 'Inger' | 39.4 | 63.8 | 69.0 | 57.4 |
| 4 | 'Jorr' | 34.5 | 37.5 | 65.3 | 45.8 |
| 5 | 'Sven' | 36.9 | 57.7 | 72.7 | 55.8 |
| 6 | 'Cozia 1' | 40.6 | 55.5 | 70.3 | 55.5 |
| 7 | 'Pesceana 7' ('Pesred') | 39.8 | 42.4 | 80.1 | 54.1 |
| 8 | 'Robeşti 1' ('Robisal') | 39.4 | 35.5 | 73.9 | 49.6 |
| 9 | 'Cozia casantă' ('Fragisal') | 30.8 | 49.0 | 60.4 | 46.7 |
| ***General Mean*** | | ***35.5*** | ***45.7*** | ***69.4*** | ***50.2*** |

The most productive genotypes proved to be: 'Inger' (57.4 t/ha), 'Sven' (55.8 t/ha), 'Cozia 1' (55.5 t/ha) and 'Pesceana 7' - 'Pesred' (54.1 t/ha). In the fourth year, some genotypes have produced high biomass yields: 80.1 t/ha ('Pesceana 7' - 'Pesred'), 73.9 t/ha ('Robeşti 1' – 'Robisal'), 72.7 t/ha ('Sven'), 71.5 t/ha ('Tordis') and 70.3 t/ha ('Cozia 1').

The biomass production of Romanian willow selection in SRC plantations type is comparable with that of cultivars introduced from other countries. Same like annual growth the average biomass yield increased since 2nd year (35.5 t/ha), 3rd year (45.7 t/ha) and 4th year (69.4 t/ha) after planting.

The yield potential of these genotypes, under the conditions from North Oltenia was 50.2 t/ha (3 years mean), similar to that recorded in SRC plantations in countries like Sweden, Poland, Hungary, etc.

Quality of harvested biomass may be influenced by the number of annual growth, average length, but also the number of annual growth with lateral shoots and the number of anticipate shoots on branches (Table 3). In the 4th year (2012) it was observed that the average number of shoots is 23.3 pcs./ plant for introduced cultivars and 22.6 pcs./ plant for the Romanian willow selections.

Table 3

Anticipate shoots issuing on annual growth for the studied *Salix* cultivars and selections

| No. | Cultivars / Selections | Number of shoots per plant (4th year - 2012) | Annual growth with anticipate shoots | | Average number of anticipate shoots per annual growth | Amplitude of anticipate shoots length (mm) |
|---|---|---|---|---|---|---|
| | | | No/plant | % | | |
| 1 | 'Tordis' | 21.0 | 3.0 | 14.3 | 2.5 | 0.3-0.6 |
| 2 | 'Tora' | 15.0 | 5.0 | 33.3 | 2.8 | 0.3-0.5 |
| 3 | 'Inger' | 27.2 | 6.7 | 19.6 | 2,6 | 0.2-0.6 |
| 4 | 'Jorr' | 26.4 | 5.3 | 17.6 | 2,1 | 0.3-0.7 |
| 5 | 'Sven' | 27.1 | 11.7 | 36.9 | 3.0 | 0.1-0.4 |
| ***Mean of introduced cvs.*** | | ***23.3*** | ***6.3*** | ***24.3*** | ***2.6*** | **-** |
| 6 | 'Cozia 1' | 20.7 | 13.3 | 64.2 | 8.0 | 0.3-0.8 |
| 7 | 'Pesceana 7' ('Pesred') | 19.0 | 12.7 | 66.8 | 10.0 | 0.2-0.8 |
| 8 | 'Robeşti 1' ('Robisal') | 24.4 | 18.3 | 67.0 | 10.0 | 0.3-0.9 |
| 9 | 'Cozia casantă' ('Fragisal') | 26.2 | 19.0 | 61.3 | 7.0 | 0.4-1.2 |
| ***Mean Romanian selections*** | | ***22.6*** | ***15.8*** | ***64.8*** | ***8.8*** | **-** |

Important differences between the two groups of genotypes are recorded regarding the number of annual growth with lateral shoots (6.3 pcs. for introduced cultivars and 15.8 pcs. for Romanian selections).

In the case of introduced willow cultivars the annual growth from the plant stumps produce anticipate lateral shoots in 14.3% cases ('Tordis') up to 36.9% cases('Sven'), while the Romanian selections had lateral shoots in 61.3% cases ('Cozia casantă' - 'Fragisal') up to 67.0% cases ('Robesti 1' - 'Robisal').

The average number of anticipated shoots per branch varies in average from 2.1 to 3.0 for the introduced cultivars and from 7.0 to 10.0 for the Romanian willow

selections. Anticipate shoots are thin in diameter (3.0 to 8.0 mm) and have lengths ranging from 0.1 to 1.2 m.

This can influence the production of biomass per ha because of a higher percentage of water in their composition.

The Romanian selections show a strong character of producing lateral shoots (64.8%) comparative with the foreign *Salix* cultivars, the difference between the two groups being 40.5% when the rainfall from April to July was 373.4 mm. In the case of willows grown into 90 liters containers and under irrigation conditions the percentage of anticipate shoots varied between 14.3% ('Pesceana 7' - 'Pesred') to 27.8% ('Cozia casantă' - 'Fragisal'), levels which are quite close to the control cultivar 'Tordis' (14.3% - 18.2%).

Table 4

Forming characteristics of the willow annual growth per plant depending on the field conditions (in SRC type plantation) and in the container culture

| *Salix* cultivars | Average number of annual growth/plant | | | Average number of annual growth with anticipate lateral shoots | | | Percentage of anticipate shoots per plant (%) | |
|---|---|---|---|---|---|---|---|---|
| | Field (non-irrigated) | Container (irrigated) | Difference from field (%) | Field (non-irrigated) | Container (irrigated) | Difference from field (%) | Field (non-irrigated) | Container (irrigated) |
| 'Tordis' (Control) | 21.0 | 22.0 | +4.8 | 3.0 | 4.0 | +33.3 | 14.3 | 18.2 |
| 'Fragisal' | 26.2 | 18.0 | -31.3 | 19.0 | 5.0 | -26.3 | 61.3 | 27.8 |
| 'Pesred' | 19.0 | 28.0 | +47.3 | 12.7 | 4.0 | -31.5 | 66.8 | 14.3 |
| 'Robisal' | 24.4 | 24.5 | +0.4 | 18.3 | 5.0 | -27.3 | 67.0 | 20.4 |

The groups of introduced willow cultivars show a pronounced genetic determinism regarding the vertical growth of branches and small number of anticipate shoots issuing even in rainfall conditions of 600-700 mm/year. The Romanian selections were very much influenced by lack of humidity and promoted annual growth with anticipate shoots.

The environmental influence is stronger than the general influence of genotype in case of local selections and therefore irrigation is needed to compensate for lack of rainfall.

## CONCLUSIONS

Under the conditions of Northern Oltenia the SRC bioenergy plantations of *Salix* achieve biomass yields of 41.7 to 57.4 t/ha, comparable to those obtained in other countries.

The Romanian selections of *Salix* achieved biomass yields of 46.7 to 55.5 t/ha, similar to the most productive foreign willow cultivars.

The biomass yields in SRC plantations grow since second year (35.5 t/ha) till forth year (69.4 t/ha).

Under non-irrigation conditions and with 373.4 mm rainfall (from April till July) on the annual growth lateral anticipated shoots form at a rate of 24.3% for introduced willow cultivars till 64.8% for the Romanian selections – in the first case an average of 2.6

anticipated shoots/branch was recorded, while in the case of Romanian selection the average recorded was 8.8 anticipated shoots/branch.

Large number of anticipated lateral shoots has negative influence on biomass yield per hectare in the case of local willow selections.

The local willow selections have high capability of issuing lateral shoots when insufficient rainfall is not supplemented by irrigation.

In terms of good growing technology and with adequate soil moisture the Romanian selections provide higher biomass yields and a quality similar to those of introduced willow cultivars, but a better resistance/tolerance to specific pests and diseases.

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# THE INFLUENCE OF ECOLOGICAL FACTORS ON THE PHYSIOLOGY OF MELISSA OFFICINALIS L. PLANTS

Buse Dragomir Luminita[1], Nicolae Ion[1]

***Keywords***:,*lemon balm, photosynthesis, essential oil*

## ABSTRACT

*Experiences aimed the weakening influence exerted by ecological factors on the physiological processes and volatile oil content at the leaves from Melissa officinalisL. plants.*

*The experiments has been made on spontaneous plants, found in the forest of ,,Silvic Ocol Corcova-,Mehedinti County, , in location with different quantity of soil water and different intensity of light.*

*The growth parameters and the developmental stage of plants influencing the physiological process and the essential oil composition of leaves*

## INTRODUCTION

*Melissa officinalis*, member of the family Lamiaceae (Labiatae) is a perennial bushy plant and is upright, reaching a height of about 1 m. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped The leaf surface is coarse and deeply veined, and the leaf edge is scalloped or toothed (Zargari, 1991).

Flowers white or pale pink consisting of small clusters of 4 to 12 blossom in the summer. It is commonly referred to as ,,lemon balm,, because of its lemon-like flavor and fragrance.

*Melissa officinalis* releases lemon-scented essential oil which contains active substances, among others: citronellal, citronellol,geranial, neral and linalool (Adenie et al. 2008; Cosge et al. 2009).

Thanks to these compounds, lemon balm is classified as an important medicinal, spice and cosmetic plant; it also exhibits sedative, antiseptic and antihistaminic activity (Nemet and Szekely, 2000; Muntean and Marian, 2005; Bahtiyarca and Cosge, 2006; Adenie et al. 2008).

Due to aromatic values, valuable pharmacological components as well as the ornamental and apicultural value of its flowers, lemon balm is often grown in vegetable and

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herb gardens (Hose M, Erman F, 1997).In literature, honey yield of lemon balm is estimated to be 32 – 150 kg/ha (Muntean,Marian 2005).

Lemon balm has a hairy root system with many lateral roots, which makes the plant more adaptable to different environmental conditions; the upper parts of the plant die at the start of winter and the new shoots re-emerge from roots at the beginning of spring (Paun M. et al, 1980).

Melissa officinalis is used in herbal medicine and is native to the eastern Mediterranean Region and western Asia (Meftahizade et al., 2010).

It is reported that the plant is mainly grown in Germany,France, Italy, Romania, Bulgaria, and North America.( Nemeth E., Szekely G., 2000) Biotic and abiotic stresses exert a considerable influence on the production of several metabolites in plants (Jaleel et al,2001, citated by Nazila A.et al, , 2011). Drought is one of the most important abiotic stress factors (Dash and Mohanthy,2001 citated by Nazila A et al, 2011), affecting plant growth and leaf photosynthesis( Flexas et al 2004 citated by Nazila A et al, 2011) and altering biochemical properties of plants.

## MATERIALS AND METHODS

The experiments has been made on spontaneous lip balm plants, found in the forest of ,,Silvic Ocol Corcova,, , in location with different conditions: 1. full sun, where the quantity of soil water soil is minimum( 20-25% from the total capacity of soil water retention); 2.moderat light and soil humidity(60-70% from the total capacity of soil water retention; 3. shade area with excess soil moisture (90-95% from the total capacity of soil water retention)

The parameters like photosynthetic rate, transpiration rate, stomatal conductance, were estimated through using the LCi portable system.

All the measurement were taken from 11 $^{00}$ to 14 $^{00}$ a.m. with the following parameters: atmospheric pressure, water gas pressure in to the chamber , photosynthetic active radiation (PAR) at leaf surface, temperature of leaf, extern temperature. The process of photosynthesis was determined for all plants studied in the 6 leaf stage, when flowering and after the flowers fell.

Assimilating pigments content was determined in 6 leaf stage using the portable chlorophyllmeter Minolta. Essential oil of leaves was extracted with alcohol. Alcoholic solution was subjected to distillation and pure volatile oil was obtained.

Determination of soil water content was done by weighing the soil sample and drying at oven at 105 $^{0}$C.

## RESULTS AND DISCUSSIONS

The process of photosynthesis was determined for all plants studied the 6 leaf stage, when flowering and after the flowers fell.

According to data (fig. 2), shows that the process is carried out at maximum intensity when flowering at moderate humidity area plants, slightly shaded. The lowest values are recorded on plants in full sun, where the quantity of water from soil is minimum(1). Values are recorded and also reduced on the plants grown in the shade area with excess soil moisture (3). To this, reducing photosynthesis is due both low light intensity, and excess of water in soil.

Fig. 1. *Melissa officinalis* L. (orig.)

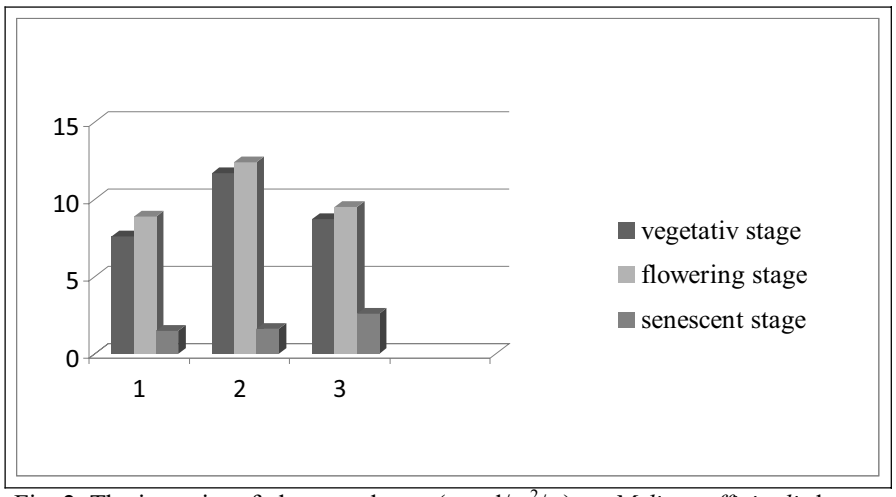


Fig. 2. The intensity of photosynthesys (µmol/$m^2$/s ) on *Melissa officinalis* leaves

The chlorophyll content

Measurements performed on mature leaves, during the blooming period, showed significant differences between total chlorophyll content of studied plants. The highest content of pigments(46 SPAD unities) had plants placed in moist and shady areas; lowest content was found in case of plant leaves placed in forest clearings, brighter(33 SPAD unities)( fig 3).

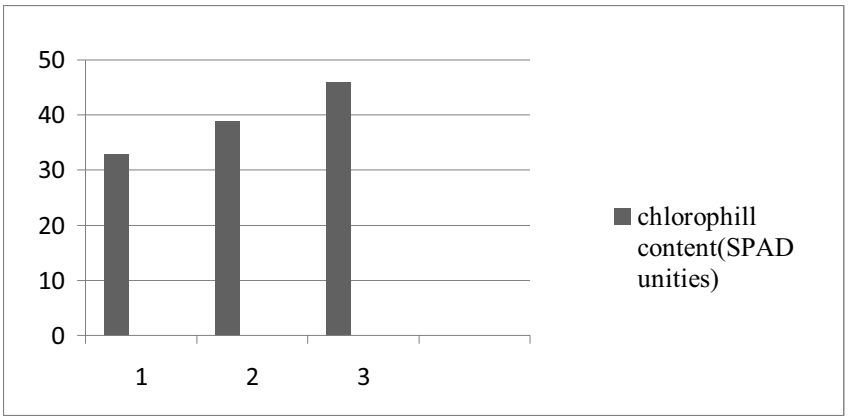


Fig.3. The chlorophyll content ( SPAD unities) on Melissa officinalis leaves

Regarding the transpiration process, it was determined the amount of water removed from plants in the three stages of development, but also on plants grown in different conditions.

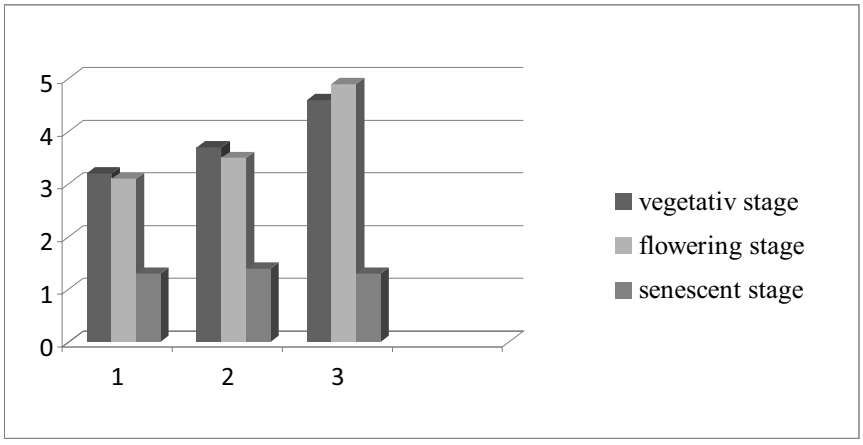


Fig. 4. The intensity of transpiration process(mmol $H_2O/m^2/s$ ) on *Melissa officinalis* leaves

As shown in the graphic the plants from wet shaded area had the highest values of transpiration in vegetative and flowering stages(4,6 - 4,9 mmol/$m^2$/s) .

In the aging phase there were not recorded significant differences of transpiration values.

The essential oil content

As a final product in the process of photosynthesis, essential oil is found in varying amounts in plants, depending on the conditions which they grew.

Environmental factors that influence the process of photosynthesis: water, light, temperature, influence the content of volatile oils.

At Melissa, essential oil content ranged from 0,01-0,05ml/g vegetal material. The lower content in essential oils was found at plants grown in the shade, in moist areas. In terms of plant vegetation stage, the minimum content in essential oils was recorded in young plants, in the vegetative phase.

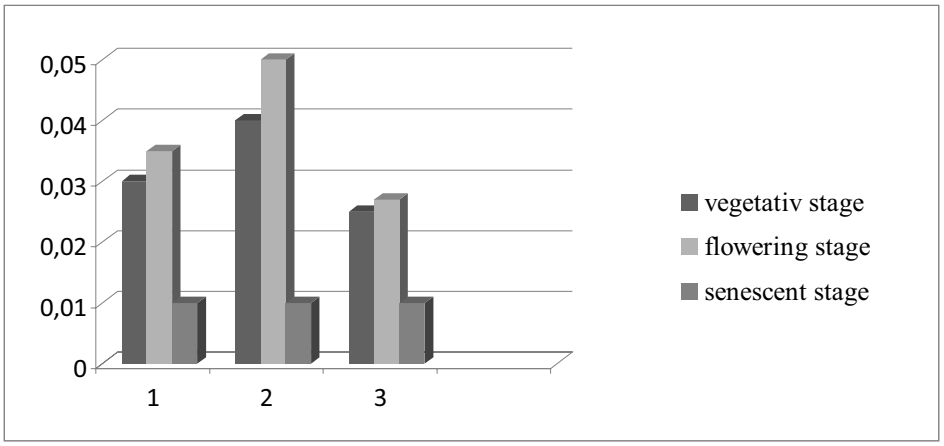


Fig. 5. The essential oil content (ml/g)

Ozturk et al (2004), citated by Moradkhani et al (2010), reported that essential oil ratio affected positively by increasing water deficit wile it was affected negatively by increasing salt concentration.

Pitarevic et al (1985) reported that a long dry season give a high oil production. Since the significant yield reduction starts at 25% water deficit.

Franz (1993) citated by Moradkhani et al (2010), reported that terminal leaves contained 30% more essential oil compared with middle stem or basal leaves.

Generally qualitative variation in oil composition is considered to be influenced by intrinsic genetic factors, were quantitative variation is influenced by extrinsic factors(Franz, 1993).

The essential rate is strongly affected by several factors such as light intensity, nutrient, temperature, plant part age, harvesting time. Essential oil rate increase with inceasing light intensity from 1000 to 1500 lx (Turhan et al (2006).

## CONCLUSIONS

- The growth parameters and the developmental stage of plants influencing the physiological process and the essential oil composition of leaves
- The process is carried out at maximum intensity when flowering, at moderate humidity area plants, slightly shaded
- The highest content of pigments(46 SPAD unities) had plants placed in moist and shady areas
-The plants from wet shaded area had the highest values of transpiration in vegetative and flowering stages

-The lower content in essential oils was found at plants grown in the shade, in moist areas.

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# THE FLORA OF DOBROGEA – A RICH SOURCE OF ORNAMENTAL PLANTS

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***Keywords****: wild flora, Dobrogea, ornamental plants*

## ABSTRACT

*The study included 82 species from the wild flora of Dobrogea, cultivated in the Botanical Garden "D. Brandza" in Bucharest. All studied species have ornamental potential and most of the plants are decorative by their flowers and habitus. Some species have ornamental fruits and foliage. Some suggestions for the types of landscape that be created using this species and multiplication methods based on their biological and ecological features are also offered in the paper.*

## INTRODUCTION

Both the Convention on Biological Diversity (CBD) and the Global Strategy for Plants Conservation acknowledge the importance of botanical gardens in the preservation of biodiversity. Thanks to their *ex situ* conservation actions, botanical gardens become sources of germplasm for rare and endangered plants and also for other plants from the spontaneous flora.

During the last decade, due to the genetic diversity decrease and climate changes, more and more countries have been turning their attention to spontaneous flora, searching for sources for the introduction of new species with ornamental value (Reddy et al. 2012, Xiao-Xian et Zhe-Kun 2005)

The study of the spontaneous flora, the finding of sources with decorative potential, and studies on their culture and multiplication are ways through which botanical gardens contribute to the *ex situ* plants' conservation.

Botanical gardens' staff has the relevant skills in order to germinate and propagate wild plants, and to ensure the successful introduction in culture of some of these species (Smith 2005).

This way, botanical gardens possess a great potential of introducing new ornamental plants, of becoming plant dissemination centres, and of providing valuable information about plants.

Historically, many botanical gardens, especially those located in the tropics, have acted as plant introduction centres and played a major role in the spread of germplasm of agricultural, industrial, forestry and ornamental plants around the world (Heywood 2011).

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International horticultural institutions and organizations also claim the necessity of introducing new species taken from the spontaneous flora into the culture. For example, The Royal Horticultural Society in Great Britain encourages the use of the spontaneous flora within gardens, but it has strict recommendations for the amateur gardeners regarding the gathering of seeds and living plants (http://www.rhs.org.uk/).

The plants with ornamental features from spontaneous flora are preferred, for having the added benefit of adaptability to the local and regional conditions (Chelariu 2011).

National literature specialised in this field of research shows the interest of the Romanian specialists on this subject, such studies being conducted in different parts of the country (Buta et al. 2009, Manda et al. 2009, Draghia et al. 2010).

This paper's aim is to propose the use as decorative plants of some species taken from the flora of Dobrogea, an area where this type of study has never been conducted. The plants from this region would best serve for cultivation in the south of our country.

Why Dobrogea? This area (including the Danube Delta) is a very important region for plant (about 2000 taxa) and habitat diversity (wetlands, dry grasslands, calcareous stony slopes, thermophilous woodlands, dunes and sand habitats etc.).

Here there are 20 endemic taxa, and over 200 rare plants, many of them included in IUCN Red List, Bern Convention, Habitats Directive (Anastasiu et Negrean 2006).

## MATERIAL AND METHOD

Observations were made on 82 taxa from the flora of Dobrogea that have been grown over time in the Botanical Garden of Bucharest in different outdoors sectors (Rare Plants, Useful Plants, Flora of Dobrogea, Mediterranean Flora) (Table 1).

The database includes the following information: species and genus names, plant attributes including life form, the type of propagation, flowering season and ornamental value (Ciocârlan 2009). These data were statistically processed with Microsoft Office software. Botanical nomenclature is in accordance with The Plant List (www.theplantlist.org). The sozological categories of species are in according to the Red Book of Vascular Plants from Romania (Dihoru & Negrean 2009).

The Botanical Garden "Dimitrie Brandza" has the specific features of plain region typical relief. It is situated on the West side of the city (North latitude of 44°24', East longitude of 26°06'), on the right side of the river Dambovița, at a low altitude (73-87 metres below sea level).

The climate is temperate continental with hot summers, especially in July and August, and very cold winters. The average annual rainfall reaches 607millimeters, and the average annual temperature reaches 11.4°C.

## RESULTS AND DISCUSSION

A total of 82 species from Dobrogea including 30 botanical families, are present in the Botanical Garden and all of them are ornamentally significant (Table 1). Among the studied species, dicots were distributed in 24 families with 60 species, monocots in 5 families with 21 species, and gymnosperm in one family with one species.

Particularly rich in wild ornamental species are the following families: Lamiaceae (11 species), Compositae (11 species), Poaceae (6), Amaryllidaceae (6 species) and Ranunculaceae (6 species).

The inventory shows that most of the studied species are perennial herbaceous (63 taxa), and only a few of them are annual herbs (3), biannual herbs (2), biannual-perennial

herbs (3), woody plants (8) and one vine. The presence of an increased number of perennials that can be used for ornamental purposes is an advantage, especially for amateur gardeners, because annual production of seedlings is not necessary.

The large number of species with Pontic origin (39 species), some of them with Balkan and Mediterranean trends, and also with Mediterranean origin (13 species) indicates the possibility of their use as ornamental plants in the lowlands of the southern part of Romania, in areas with a mild microclimate.

The analysis of the ecological characteristics of the species highlights the predominance of taxa with medium to large requirements in terms of temperature, thus enabling the identification of species that can be grown not only in the lowlands of the south, but also in hillsides, some also developing well in the sublevel of the sessile oak.

Regarding soil moisture requirements, there prevail the xerophytes and the xero-xeromezophytes, which indicates the possibility of using them even in unirrigated gardens and parks, these species being adapted to long periods of dryness, living on dry to dry-moist soils.

Among the studied species there are some with special requirements to the environmental conditions, some of them living on calcareous soils (*Scutellaria orientalis*, *Agropyron brandzae*, *Potentilla emilli-popii*, *Asphodeline lutea*) and others living on sands (*Leymus racemosus*, *Ephedra distachya*, *Helichrysum arenarium*, *Crambe maritima*).

Many wild plants have ornamental parts and we divided our studied species into five main types: with ornamental habitus (38 species – 29%), flowers (70 species – 54%), fruits (10 species – 8%), seeds (one specie – 1%) and ornamental foliage (10 species – 8%), but some of them are decorative by many different features at the same time (Fig. 1).

A high percentage of these species (70 species – 85.36%) has decorative flowers, some of them being at the same time decorative by flowers and habitus (29 species – 35.36%) or by habitus and the form and colour of the leaves (7 species – 8.53%), representing a very good choice to decorate the garden all year round.

Regarding those species with decorative flowers, it is required a classification according to the blooming period, which may be useful in the selection of species in the initial stages of planning the garden.

Thus, the species can be grouped into: spring plants (29 species), summer plants (46 species) and autumn plants (10 species) (Table 2). In the autumn category we also included the species with decorative fruits.

The study of the means of propagation showed that in the conditions available at the Botanical Garden from Bucharest, most of the species reproduce both by seeds and vegetative, by dividing the bush.

This constitutes a great advantage for the Botanical Garden, especially because it is only allowed to collect plant materials from nature (plants or seeds) with the approval of the environment protection authorities. Thus, botanical gardens can produce vegetal material which can then be easily multiplied by gardeners through division of bushes.

We note that among the selected species there are 25 taxa which are included in the Red Book of Vascular Plants from Romania (Dihoru & Negrean 2009), threatened to various degrees as follows: 6 critically endangered taxa (*Gymnospermium altaicum*, *Ornithogalum comosum*, *Achillea clypeolata*, *Achillea leptophylla*, *Asyneuma anthericoides*, *Onosma taurica*), 5 endangered taxa (*Salvia ringens*, *Crocus pallasii*, *Asphodeline lutea*, *Crambe maritima*, *Euphorbia myrsinites*), 13 vulnerable taxa (*Dianthus nardiformis*, *Centaurea napulifera*, *Crocus chrysanthus*, *Galanthus plicatus*, *Adonis volgensis*, *Astragalus cornutus*, *Koeleria lobata*, *Opopanax chironium*, *Potentilla emilli-popii*, *Agropyron brandzae*, *Paeonia tenuifolia*, *Jasminum fruticans*, *Satureja coerulea*) and one taxon with low risk (*Iris sintenisii*).For this reason, encouraging the cultivation of

these species would also contribute both, to the promotion and *ex situ* conservation of native species.

The investigated plants could be used in following types of landscape:

- ❖ calcareous rocks gardens - *Asphodeline lutea*, *Potentilla emilli-popii*, *Agropyron brandzae*, *Scutellaria orientalis*
- ❖ rocks gardens - *Allium flavum*, *Centaurea napulifera*, *Centaurea marschalliana*, *Achillea coarctata*, *Asyneuma anthericoides*, *Convolvulus cantabricus*, *Crocus* spp., *Dianthus nardiformis*, *Euphorbia myrsinites*, *Iris pumila*, *Satureja coerulea*, *Thymus zygioides*, *Sedum urvillei*
- ❖ groups at the margins of arborescent massifs - *Nectaroscordum siculum*, *Campanula persicifolia*, *Gymnospermium altaicum*, *Galanthus elwesii*, *Galanthus plicatus*, *Paeonia peregrina*
- ❖ groups in lawns - *Achillea coarctata*, *Achillea leptophylla*, *Adonis vernalis*, *Iris sintenisii*, *Leymus racemosus*, *Paeonia tenuifolia*, *Salvia* spp., *Tanacetum corymbosum*
- ❖ isolated in lawns - *Koeleria lobata* , *Opopanax chironium*, *Ruta graveolens*, *Stipa capillata.*

## CONCLUSIONS

We propose the introduction in culture of wild ornamental species from Dobrogea as an alternative method to maintain the diversity of the wild species and conserve the rare and endangered species of ornamental interest. They could be used to decorate the rock gardens, the edge of arborescent masiffs, the open places.

## ACKNOWLEDGEMENTS

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Table 1

Inventory of wild ornamental plants from Dobrogea cultivated in the Botanical Garden "D. Brandza"

| Taxon | Life forms | Propagation | Blooming time | Decorative elements |
|---|---|---|---|---|
| Achillea clypeolata Sm. | Perennial herbs | plant division | VI-VII | habitus, foliage, flowers |
| Achillea coarctata Poir | Perennial herbs | plant division | VI-VII | habitus, foliage, flowers |
| Achillea leptophylla M.Bieb. | Perennial herbs | plant division | VI-VII | flowers |
| Adonis vernalis L. | Perennial herbs | seeds, plant division | IV-V | flowers |
| Adonis volgensis Steven ex DC. | Perennial herbs | seeds, plant division | IV-V | flowers |
| Agropyron brandzae Pantu & Solacolu | Perennial herbs | seeds, plant division | V-VII | habitus, foliage, spikes |
| Agropyron cristatum subsp. pectinatum (M.Bieb.) B.Fedtsch. | Perennial herbs | seeds, plant division | V-VII | habitus, spikes |
| Ajuga chamaepitys (L.) Schreb. | Annual herbs | seeds | V-VIII | habitus, flowers |
| Allium flavum L. | Perennial herbs | bulbs, seeds | VII-VIII | flowers |
| Allium saxatile M.Bieb. | Perennial herbs | bulbs, seeds | VI-IX | flowers |
| Anchusa azurea Mill. | Perennial herbs | seeds | V-VII | flowers |
| Anemone sylvestris L. | Perennial herbs | seeds, plant division | V-VI | flowers |
| Asparagus verticillatus L. | Perennial herbs | seeds, plant division | V-VI | habitus, fruits |
| Asphodeline lutea (L.) Rchb. | Perennial herbs | seeds | IV-V | habitus, flowers |
| Aster oleifolius Wagenitz | Perennial herbs | seeds, plant division | VII-IX | habitus, foliage, flowers |
| Astragalus cornutus (Pall.) Kunze | Perennial herbs | seeds, plant division | VI-VII | flowers |
| Asyneuma anthericoides (Janka) Bornm. | Perennial herbs | seeds | VII-VIII | flowers |
| Beta trigyna Waldst. & Kit. | Perennial herbs | seeds, plant division | VI-VII | habitus |
| Campanula persicifolia L. | Perennial herbs | seeds | VI-VIII | flowers |

| | | | | |
|---|---|---|---|---|
| Centaurea marschalliana Spreng. | Perennial herbs | seeds, plant division | V | habitus, flowers |
| Centaurea napulifera Rochel | Perennial herbs | stolons, seeds | IV-V | flowers |
| Cerasus mahaleb Mill. | Trees or large shrubs | seeds | IV-V | flowers, fruits |
| Convolvulus cantabricus L. | Perennial herbs | seeds | V-VII | habitus, flowers |
| Cotinus coggygria Scop. | Trees | seeds | V-VI | foliage, flowers |
| Crambe maritima L. | Perennial herbs | seeds | V-VI | foliage, flowers |
| Crocus chrysanthus (Herb.) Herb. | Perennial herbs | seeds | II-IV | flowers |
| Crocus pallasii Goldb. | Perennial herbs | seeds | IX-X | flowers |
| Crocus reticulatus Steven ex Adam | Perennial herbs | seeds | II-III | flowers |
| Dianthus nardiformis Janka | Perennial herbs | seeds | VI-VIII | habitus, flowers |
| Echinops ritro L. subsp. ruthenicus (M.Bieb.) Nyman | Perennial herbs | seeds, plant division | VII-VIII | habitus, flowers |
| Ephedra distachya L. | Shrubs | seeds | VI-VII | habitus, seeds |
| Euphorbia myrsinites L. | Perennial herbs | seeds, plant division | VI-VII | habitus, flowers |
| Galanthus elwesii Hook.f. | Perennial herbs | bulbs, seeds | II-III | flowers |
| Galanthus plicatus M.Bieb. | Perennial herbs | bulbs, seeds | II-III | flowers |
| Glaucium corniculatum (L.) Curtis | Perennial herbs | seeds | VI-VIII | flowers |
| Gymnospermium altaicum Pall. | Perennial herbs | seeds | IV | flowers |
| Helichrysum arenarium (L.) Moench | Perennial herbs | seeds, plant division | VII-IX | habitus, flowers |
| Hyacinthella leucophaea (K.Koch) Schur | Perennial herbs | bulbs, seeds | III-IV | flowers |
| Hypericum elegans Stephan ex Willd. | Perennial herbs | seeds, plant division | VI-VII | flowers |
| Iris pumila L. | Perennial herbs | seeds, rhyzomes | IV-V | flowers |
| Iris sintenisii Janka. | Perennial herbs | seeds, rhyzomes | V | flowers |
| Isatis tinctoria L. | Biannual-perennial herbs | seeds | V-VII | habitus, flowers, fruits |

| | | | | |
|---|---|---|---|---|
| Jasminum fruticans L. | Shrubs | seeds, plant division | V-VI | flowers |
| Koeleria lobata (M.Bieb.) Roem. & Schult. | Perennial herbs | seeds, plant division | V-VII | habitus, spikes |
| Leymus racemosus (Lam.) Tzvelev | Perennial herbs | seeds, plant division | VI-VII | habitus, foliage, spikes |
| Limonium latifolium O.Kuntze | Perennial herbs | seeds | VII-IX | habitus, flowers |
| Nectaroscordum siculum (Ucria) Lindl. | Perennial herbs | bulbs, seeds | V | flowers |
| Onosma taurica Pall. | Perennial herbs | seeds | V-VI | flowers |
| Opopanax chironium (L.) Koch subsp. bulgaricum (Velen.) Ciocârlan | Perennial herbs | seeds | VI_VII | habitus |
| Orchis purpurea Huds. | Perennial herbs | tubers, seeds | V-VI | flowers |
| Orlaya grandiflora Hoffm. | Annual herbs | seeds | V-VII | flowers |
| Ornithogalum comosum L. | Perennial herbs | bulbs, seeds | V | flowers |
| Oryzopsis holciformis (M.Bieb.) Hack. | Perennial herbs | seeds, plant division | V-VI | habitus |
| Paeonia peregrina Mill. | Perennial herbs | seeds, plant division | V-VI | habitus, flowers, fruits |
| Paeonia tenuifolia L. | Perennial herbs | seeds, plant division | IV-V | habitus, flowers, fruits |
| Paliurus spina-christi Mill. | Shrubs | seeds | V-VIII | foliage, fruits |
| Periploca graeca L. | Vine | seeds | IV-VI | habitus, fruits |
| Potentilla emilli-popii Nyar. | Perennial herbs | seeds | V-VI | habitus, flowers |
| Pulsatilla montana Hoppe. | Perennial herbs | plant division | III-IV | flowers, fruits |
| Ranunculus illyricus L. | Perennial herbs | seeds | V-VI | habitus, flowers |
| Ranunculus oxyspermus Willd. | Perennial herbs | seeds | V-VI | flowers |
| Rosa gallica L. | Shrubs | suckers | VI-VII | flowers |
| Ruta graveolens L. | Perennial herbs | seeds, plant division | VI-VIII | habitus, foliage, flowers, fruits |
| Salvia aethiopis L. | Biannual-perennial herbs | seeds, plant division | VI-VIII | flowers |
| Salvia austriaca Jacq. | Perennial herbs | seeds, plant division | IV--VI | habitus, flowers |

| | | | | |
|---|---|---|---|---|
| Salvia nemorosa L.s.l. | Perennial herbs | seeds, plant division | VI-VIII | flowers |
| Salvia nutans L. | Perennial herbs | seeds, plant division | V-VII | flowers |
| Salvia ringens Sm. | Perennial herbs | seeds, plant division | V-VII | flowers |
| Salvia sclarea L. | Biannual-perennial herbs | seeds, plant division | VI-VII | habitus, flowers |
| Satureja coerulea Janka | Subshrus | seeds, plant division | VII-IX | habitus, flowers |
| Scutellaria orientalis L. | Subshrus | seeds, plant division | V-VII | habitus, flowers |
| Sedum urvillei DC. | Perennial herbs | plant division | V-VII | habitus, flowers |
| Seseli tortuosum L. | Biannuals herbs | seeds | VII-VIII | habitus |
| Smyrnium perfoliatum L. | Biannuals herbs | seeds | IV-VI | habitus |
| Stipa capillata L. | Perennial herbs | seeds, plant division | VII-VIII | habitus, fruits |
| Tanacetum corymbosum L. | Perennial herbs | seeds, plant division | VI-VIII | flowers |
| Tanacetum millefolium (L.) Tzvelev | Perennial herbs | seeds, plant division | VII-VIII | foliage, flowers |
| Taraxacum erythrospermum Andrz.ex Besser | Perennial herbs | seeds | IV-V | flowers |
| Teucrium capitatum L. | Perennial herbs | seeds, plant division | VII-VIII | habitus, foliage, flowers |
| Thymus zygioides Griseb. | Perennial herbs | seeds, plant division | V-VII | habitus, flowers |
| Vicia serratifolia Jacq. | Annual herbs | seeds | V-VI | flowers |

Table 2

Wild ornamental plants from Dobrogea classified according to the blooming period

| Type of plants by blooming time | Botanical name |
|---|---|
| Spring plants | *Adonis vernalis*, *Adonis volgensis*, *Anemone sylvestris*, *Asparagus verticillatus*, *Asphodeline lutea*, *Centaurea marschalliana*, *Centaurea napulifera*, *Cerasus mahaleb*, *Crocus chrysanthus*, *Crocus reticulatus*, *Galanthus elwesii*, *Galanthus plicatus*, *Gymnospermium altaicum*, *Hyacinthella leucophaea*, *Iris pumila*, *Iris sintenisii*, *Nectaroscordum siculum*, *Onosma taurica*, *Orchis purpurea*, *Ornithogalum comosum*, *Paeonia peregrina*, *Paeonia tenuifolia*, *Potentilla emilli-popii*, *Pulsatilla montana*, *Ranunculus illyricus*, *Ranunculus oxyspermus*, *Salvia austriaca*, *Taraxacum erythrospermum*, *Vicia serratifolia* |
| Summer plants | *Achillea clypeolata*, *Achillea coarctata*, *Achillea leptophylla*, *Agropyron brandzae*, *Agropyron cristatum*, *Ajuga chamaepitys*, *Allium flavum*, *Allium saxatile*, *Anchusa azurea*, *Aster oleifolius*, *Astragalus cornutus*, *Asyneuma anthericoides*, *Beta trigyna*, *Campanula persicifolia*, *Convolvulus cantabricus*, *Cotinus coggygria*, *Crambe maritima*, *Dianthus nardiformis*, *Echinops ritro*, *Ephedra distachya*, *Euphorbia myrsinites*, *Glaucium corniculatum*, *Helichrysum arenarium*, *Hypericum elegans*, *Isatis tinctoria*, *Jasminum fruticans*, *Koeleria lobata*, *Leymus racemosus*, *Limonium latifolium*, *Orlaya grandiflora*, *Pulsatilla montana*, *Rosa gallica*, *Ruta graveolens*, *Salvia aethiopis*, *Salvia austriaca*, *Salvia nemorosa*, *Salvia nutans*, *Salvia ringens*, *Salvia sclarea*, *Satureja coerulea*, *Scutellaria orientalis*, *Sedum urvillei*, *Tanacetum corymbosum*, *Tanacetum millefolium*, *Teucrium capitatum*, *Thymus zygioides* |
| Autumn plants | *Aster oleifolius*, *Cerasus mahaleb*, *Crocus pallasii*, *Isatis tinctoria*, *Paeonia peregrina*, *Paeonia tenuifolia*, *Paliurus spina-christi*, *Periploca graeca*, *Stipa capillata, Asparagus verticillatus* |

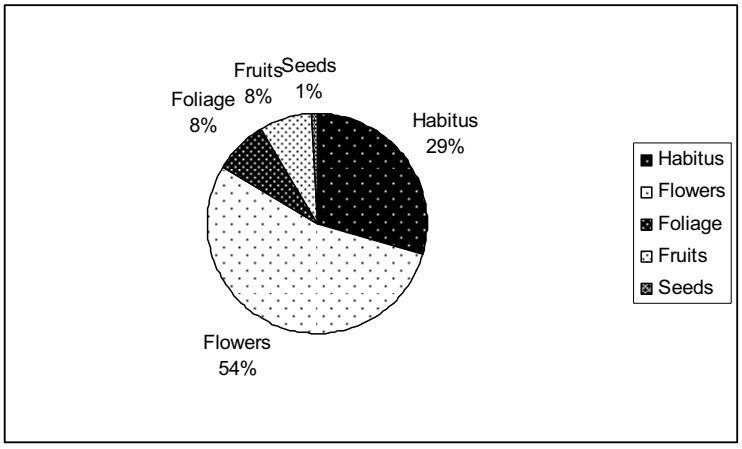


Fig. 1. Number of wild ornamental plants classified according to the ornamental parts

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✓ *Ingineria mediului*

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# PHENOTIPIC DIVERSITY EVALUATION OF SOME BULB CHARACTERS OF LOCAL SHALLOT (*Allium ascalonicum*) LANDRACES FROM SALINE SOILS IN BANAT AREA

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***Keywords****: shallot, local landraces, bulb traits, UPGMA clustering*

## ABSTRACT

*Though the salt tolerance in cultivated species is low, there are some genotypes which present an acceptable level of adaptability to moderate saline conditions. In legumes, these genotypes are represented especially by local landraces breeded over the time by local farmers from areas with saline soils. Identification of these cultivars represents a priority in breeding programs of vegetables all over the world. The paper aims to assess phenotypic diversity bulb character (shape, diameter, height and weight) in 16 shallots landraces grown in saline areas of southwestern Timis Count. The obtained results prove the existence of a large bulb discharged phenotypic characters, even between genotypes from the same location (Dolat and Rudna) which suggests that adaptation to specific environmental conditions have been associated with different morphological features of bulbs.*

## INTRODUCTION

Local shallot landraces are used in Europe, USA and Asia due to their particular taste, persistent leaves and multiple healing effects (Fritsch and Friesen, 2002; Brewster, 2008; Susheela 2007). Growers use mostly local landraces with a high adaptability to specific environmental conditions determined by the abiotic stress factors. Due to its special culinary properties and to high ecological adaptability (tolerance to drought, salinity and powerful solar insolation), shallot (*Allium ascalonicum*) is cultivated in many peasant farms mostly for consumption or to be sold.

Saline soils affect around 15% of the agricultural lands in Timis county. There are two main geographical areas affected by saline soils: the South-Western area - Livezile, Cruceni, Foeni, Uivar, Cenei, Săcălaz, Peciu-Nou Ciacova, and the North-Western area - Dudeştii-Vechi, Beba-Veche, Cenad, Sînicolaul-Mare, Sînpetru-Mare and Teremia Mare. In these area the cultivation of onion in houshold system and micro-farms is traditional but there is also a great pressure by introducing of new commercial varities for cultivation.

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Nevertheless, local landraces are still cultivated because they have special qualities and are accepted by local markets.

Evaluation of local onion lines have been carried out all over the world. Most of these characterizations are based either on morphological, agronomical or physical and chemical measurements (Rivera Martinez et.al 2005).

During summer 2012 a group of researchers from BUASVM Timisoara have collected bulbs from local shallot landraces from areas affected by salinity in Banat region, activity wich was financed from the project "The screening of salinity tolerance of some local vegetable landraces in order to conserve the genetic potential and biodiversity" through PN-II-PT-PCCA-2011. This paper presents some of the results regarding the phenotypic diversity of some bulb characters at 16 local shallot landraces collected from South-Western aline area of Timis county.

## MATERIAL AND METHODS

The studied biological material was composed of 16 shallot landraces collected from localities of Timis county with saline soils. The experimental design was a randomized complete block (RCB) with three replications. From each plot 20 bulbs were evaluated for the following traits: bulbs height (BH); bulbs diameter (BD); bulbs weight (BW); shape index (SI).

The data were analyzed by Jaccard similarity coefficients, UPGMA cluster analysis (Fielding, 2007), principal components, ANOVA (Ciulca , 2006). The distance matrix was used for cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA), with the Neighbor program of the Phylip package, version 3.5c. To make possible the display in a single graph of the performance of each genotype for each of the five traits, the basic principle of the biplot technique developed by Gabriel (1971) and GGE biplot method developed by Yan et. al. (2000) were used.

## RESULTS AND DISCUSSIONS

The differences between variants marked with different letters are considered to be significant. Regarding the bulb diameter of the populations, we observed that there is a variation amplitude of 3.50 cm, which is associated with a medium-high inter-genotypic variability (20.23 %), with limit values of 3,40 cm at Rudna 304 and 6.90 cm at Dolat 1261 populations. Dolat 1261 showed a significantly higher ($p<5\%$) bulb diameter compared to the rest of the populations, which did not differ significantly ($p<5\%$).

The character of bulb height presented a medium inter-population variability, with the lowest values of 3.50 cm for Dolat 198 and the highest, for Rudna 101 (6.50 cm). The variation amplitude was 3 cm. At $p<5\%$, Rudna 101 population presented a significantly superior height than the most of the other populations, with the exception of Dolat 1261, Dolat 1262, Giera 6, Rudna 124, Sanmartin S. 108 and Uivar 305. Low values for the bulb height character were observed at populations Dolat 198, Dolat 244, Toager 1 and Toager 207. For the character bulb weight, the variability amongst the populations was very high (39,64%), associated with an amplitude of 64,58 g. The population Dolat 1261 had the highest values, which were also significantly higher at $p<5\%$ than the most of the other populations. We could also observe high values of bulb weight at populations Sanmartinu

S. 108 and Rudna 101, which had bulbs with average weight over 80 g. The smallest bulbs were developed by populations Toager 1, Dolat 198 and Toager 207 (below 30 g).

Table 1

Mean values of the studied bulb traits in shallot landraces

| | Diameter (cm) | Height (cm) | Weight(g) | Shape index |
|---|---|---|---|---|
| Dolat 1261 | 6.90±0.10a | 5.45±0.15abcde | 88.76±2.58a | 0.79±0.01f |
| Dolat 1262 | 4.27±0.07b | 6.00±0.46ab | 49.71±4.05c | 1.41±0.11abc |
| Dolat 198 | 3.60±0.10b | 3.50±0.29g | 25.11±4.80c | 0.97±0.06def |
| Dolat 244 | 4.17±0.23b | 3.93±0.18f | 36.15±3.64c | 0.95±0.08ef |
| Foieni 343 | 3.57±0.03b | 4.70±0.15bcdefg | 42.56±3.51c | 1.32±0.06abcde |
| Giera 6 | 3.67±0.03b | 5.70±0.65abcd | 53.51±5.83abc | 1.55±0.17ab |
| Livezile 498 | 3.87±0.13b | 4.47±0.07cdefg | 40.96±1.71c | 1.16±0.04cdef |
| Rudna 101 | 3.85±0.15b | 6.50±0.40a | 81.43±5.58abc | 1.69±0.17a |
| Rudna 124 | 4.20±0.15b | 5.13±0.23abcdef | 53.89±2.29abc | 1.23±0.09bcde |
| Rudna 304 | 3.40±0.10b | 4.50±0.17cdefg | 40.67±0.80c | 1.33±0.09abcd |
| Sanmartinu S.180 | 4.45±0.15b | 5.90±0.10abc | 82.47±4.72ab | 1.33±0.07abcd |
| Uivar 305 | 4.00±0.50b | 5.40±0.40abcdef | 52.25±3.23abc | 1.36±0.07abc |
| Uivar 306 | 3.75±0.25b | 4.40±0.10defg | 39.85±2.73c | 1.18±0.11bcde |
| Toager 1 | 3.43±0.07b | 3.67±0.17fg | 24.18±2.99c | 1.07±0.04cdef |
| Toager 29 | 3.87±0.13b | 4.97±0.27bcdefg | 50.79±5.89bc | 1.28±0.05bcdef |
| Toager 207 | 3.73±0.13b | 3.97±0.15efg | 29.98±3.65c | 1.06±0.02cdef |
| $LSD_{5\%}$ | 1.36 | 1.49 | 32.73 | 0.38 |
| $LSD_{1\%}$ | 1.84 | 2.00 | 44.08 | 0.52 |
| $LSD_{0,1\%}$ | 2.44 | 2.66 | 58.44 | 0.69 |

Regarding the bulbs' shape, considering the shape index, most of the populations, with only three exceptions, presented elongated shape. Inter-population variability was medium, similar to the values for bulb height. Populations Rudna 101 and Giera 6 had the longest bulbs, meanwhile populations Dolat 1261, Dolat 198 and Dolat 244 had ovoid-oblate shaped bulbs.

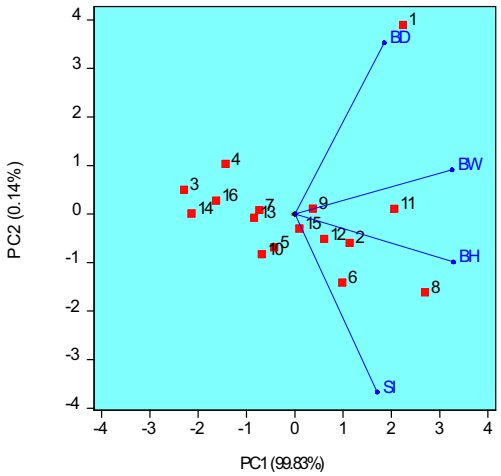


Fig. 1.Biplot for the studied shallot landraces and bulbs traits

The Biplot diagram (Fig. 1) based on the two main components expresses 99.97% of the variability for the four characters. Based on the genotypes' position in regard to the vectors of the different characters, it was expressed their performance. Thus it can be observed that Dolat 1261, Rudna 101 and Sanmartin S. 180 present high values for the quantitative characters, such as height, diameter and weight. Values higher for these three characters than the mean were observed at populations Dolat 1262, Giera 6, Rudna 124, Toager 29 and Uivar 305. For Rudna 101 and Giera 6 populations, the quantitative values of bulbs were associated with the elongated shape.

Populations Dolat 198, Dolat 244, Toager 1 and Toager 207 presented reduced dimensions of the bulbs, associated with oblate shape.

Populations Foieni 343, Rudna 304, Livezile 498 and Uivar 306 presented medium dimensions and ovoid shaped bulbs.

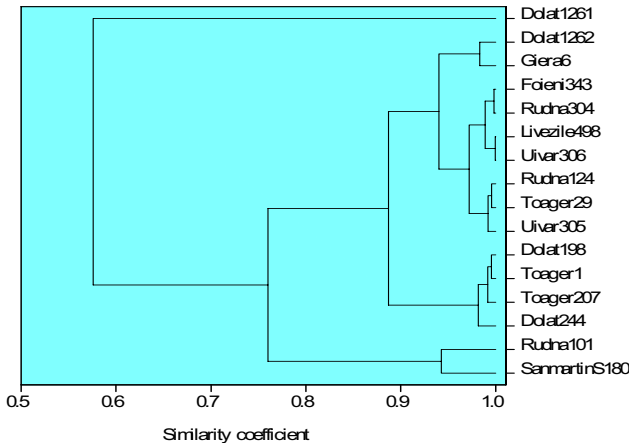


Fig. 2. UPGMA clustering of shallot landraces for studied bulbs traits

Considering the phenotypic homogeneity of the 16 shallot populations, we constructed a dendrogram using the method of clusters' average. The dendrogram shows the existence of two main clusters, one of them containing only one member, population Dolat 1261, which manifests an average phenotypic diversity of 42% compared to the rest of the populations.

The second cluster is composed of three sub-clusters: the first contains nine populations with an average similitude of 96% compared to the other seven populations, and contains sub-groups with higher (Dolat 1262, Giera 6, Rudna 124, Toager 29 and Uivar 305) or lower (Foieni 343, Rudna 304, Livezile 498 and Uivar 306) values of bulbs. The second cluster is composed of populations Dolat 198, Dolat 244, Toager 1 and Toager 207, with a similitude of 96,5%. This cluster contains populations with smaller and oblate bulbs. The third sub-cluster includes the populations Rudna 101 and Sanmartin S., which have an average similitude of 93 % and present larger bulbs.

Table 2

Analysis of variance for shallot landraces concerning the bulb traits

| No. | Landraces | Between groups | | Within groups | | F Test |
|---|---|---|---|---|---|---|
| | | SS | DF | SS | DF | |
| 1 | Dolat1261 | 5339.99 | 1 | 20.38 | 2 | 523.95** |
| 2 | Dolat1262 | 1574.38 | 1 | 10.75 | 2 | 292.99** |
| 3 | Dolat198 | 376.99 | 1 | 4.44 | 2 | 169.72** |
| 4 | Dolat244 | 823.36 | 1 | 6.44 | 2 | 255.88** |
| 5 | Foieni343 | 1162.10 | 1 | 5.92 | 2 | 392.52** |
| 6 | Giera6 | 1865.26 | 1 | 8.61 | 2 | 433.15** |
| 7 | Livezile498 | 1071.25 | 1 | 6.22 | 2 | 344.45** |
| 8 | Rudna101 | 4495.01 | 1 | 11.61 | 2 | 774.47** |
| 9 | Rudna124 | 1902.85 | 1 | 8.30 | 2 | 458.60** |
| 10 | Rudna304 | 1059.94 | 1 | 5.18 | 2 | 409.14** |
| 11 | Sanmartin S.180 | 4630.72 | 1 | 10.91 | 2 | 849.09** |
| 12 | Uivar305 | 1776.09 | 1 | 8.42 | 2 | 422.02** |
| 13 | Uivar306 | 1012.37 | 1 | 5.80 | 2 | 349.18** |
| 14 | Toager 1 | 345.29 | 1 | 4.13 | 2 | 167.25** |
| 15 | Toager 29 | 1686.26 | 1 | 7.18 | 2 | 469.84** |
| 16 | Toager 207 | 549.18 | 1 | 5.22 | 2 | 210.49** |

Considering the results presented in Table 2, it can be concluded that populations Dolat 1261, Rudna 101 and Sanmartin S 180 give the highest differences between the bulb traits, having significant ($p<1\%$) contributions to total variability. The smallest contribution to the total variability had the populations Toager 1, Dolat 198 and Toager 207. Regarding the analysis of variance for the quantitative traits, significantly higher values were measured for bulb weight, which manifests a high differentiation capacity between the populations of the same cluster, and also between the populations of the other clusters. The lowest variability between the populations was observed for the bulb shape trait.

Table 3

Analysis of variance for bulb traits of shallot landraces

| No. | Landraces | Between groups | | Within groups | | F Test |
|---|---|---|---|---|---|---|
| | | SS | DF | SS | DF | |
| 1 | Bulb diameter | 3.85 | 1 | 6.20 | 14 | 8.69* |
| 2 | Bulb height | 4.17 | 1 | 7.76 | 14 | 7.53* |
| 3 | Bulb weight | 4446.67 | 1 | 1333.25 | 14 | 46.69** |
| 4 | Shape index | 0.01 | 1 | 0.79 | 14 | 0.10 |

## CONCLUSIONS

The study concluded that there is a considerable diversity for the bulb traits, which can be useful in plant breeding programs. It was also observed that there is a great diversity between the populations deriving from the same place, such as Dolat and Rudna. This suggests that the adaptation to specific environmental conditions was associated with different morphological traits of bulbs.


## ACKNOWLEDGEMENT

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number PN-II-PT-PCCA-2011-3.1-0965.
Special thanks to all persons from villages which were very kind to let to visit their gardens and supplied us with the necessary plant material.


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# STUDY REGARDING PHENOTYPIC VARIABILITY OF SOME TOMATOES LOCAL LANDRACES CULTIVATED IN BANAT AREA

Camen D.[1], Sumalan R[1]., Beinsan Carmen[1], Suciu Laura[1], Popescu Ioana[1], Viliga F.[1]

***Keywords*:** *tomato, variability, phenotypic, characterization*

## ABSTRACT

*The present study was conducted at Banat University of Agricultural Sciences and Veterinary Medicine from Timisoara during summer 2012 seasons. The phenotypic correlation was analyzed over all the studied characters of tomato cultivated in the western part of Romania. For this purpose expeditions were organized collection of biological material in Timis county. Tomatoes collected were framed by fruit color measure then the main biometric characteristics: fruit height, fruit diameter and fruit weight. After framing according to the fruit colors have identified four color groups: red, purple, yellow and pink. After the biometric determinations was found that there is a large genetic variability manifested phenotypically in tomato fruits collected mainly influenced by pedo-climatic conditions.*

## INTRODUCTION

Tomatoes are one of the main crops in the vegetable garden because of the fruit which contains significant amounts of nutrients (2). Tomatoes nutritional value is given by the high content of vitamins, sugars, minerals, amino acids and organic acids in fruits both fresh and after processing (6). Although heat energy is low compared to other foods (90-176 calories), tomatoes by their content meet an important catalytic role in the metabolism of human body (3, 8). Tomatoes are now eaten freely throughout the world, and their consumption increases, being good for the heart, among others. Tomatoes contain lycopene, one of the most powerful natural antioxidant (1). Some studies have proved that lycopene, especially in cooked tomatoes, helps prevent prostate cancer (www.whfoods.com) but other studies contradict these statements.

Lycopene improves the ability to protect skin against harmful UV rays (http:// news.bbc.co.uk). Natural genetic variation in tomatoes and their wild relatives provides a "treasure" of genes that produce lycopene, anthocyanin carotene and other antioxidants (4, 5). Some tomato varieties are available with double the normal amount of vitamin C, 40 times more vitamin A than normal (97L97), high levels of anthocyanin (P20 Blue) and 2 to 4 times more amount of lycopene (numerous cultivars are available that have specific genes for intense carmine color) (7).

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## MATERIALS AND METHODS

In the experiment were collected and studied 128 tomato genotypes all grown in Banat area. After collection they were placed in Timisoara USAMVB's collection and the phenotypic and biometric characterization was also made. The collected tomatoes were divided in four color groups: red, pink, purple and yellow.

Traits of individual plant were measured on ten guarded fruits in each location. The following traits were measured:

1- Fruits fresh weight (g).

4- Fruits height (cm)

5- Fruits diameter (cm)

The results were statistically processed determining the average and standard deviation.

## RESULTS AND DISCUSSIONS

After establishing the four color groups the 128 collected genotypes were distributed as follows:

- 113 genotypes showed red color
- 4 genotypes showed pink color
- 2 genotypes showed purple color
- 9 genotypes showed yellow color

Table 1

Experimental results regarding phenotype characteristics of tomatoes - red group

| Local landraces | Type/Color | Height | Diameter | Weight |
|---|---|---|---|---|
| Cherestur 60 | big/red | 5.15±1.34 | 8.95±0.07 | 267.1±72.41 |
| Periam 48 | lengthen/red | 5.87±0.64 | 4.37±0.25 | 64.73±4.36 |
| Periam 221 | red | 6.53±0.93 | 6.5±0.36 | 167.01±10.62 |
| Periam 48 | red | 5.1±1.22 | 6.97±1.25 | 173.13±106.12 |
| Cheglevici 161 | red | 6.1±0.71 | 8.85±0.21 | 276.96±49.02 |
| Cheglevici 165 | red | 5.45±0.35 | 7.45±0.35 | 237.815±19.74 |
| Sâmpetru Mare 354 | red | 7.93±1.53 | 4.87±0.71 | 117.09±19.7 |
| Sâmpetru Mare 354 | lengthen/red | 5.53±0.67 | 6.3±0.36 | 167.20±14.05 |
| Lovrin 189 | red | 6.9±0.44 | 10.5±0.46 | 425.02±98.41 |
| Lovrin 1169 | red | 6.1±0.26 | 6.73±0.15 | 198.32±26.13 |
| Cherestur 60 | small/red | 4.17±0.51 | 5.87±0.32 | 98.08±29.05 |
| Dudestii Vechi 883 | red | 5.43±0.55 | 6.2±0.3 | 144.47±37.4 |
| Dudestii Vechi 883 | small/red | 6.6±0.87 | 3.87±0.23 | 56.99±13.59 |
| Pordeanu 18 | red | 5.83±0.72 | 7.53±1.33 | 150.8±145.99 |
| Dudestii Vechi 883 | red | 6.1±0.66 | 7.67±0.76 | 226.99±63.46 |
| Beba Veche 350 | red | 3.85±0.21 | 3.95±0.07 | 38.82±2.7 |

| | | | | |
|---|---|---|---|---|
| Lovrin 189 | big/red | 5.13±0.23 | 6.63±0.35 | 153.08±12.96 |
| Lovrin 189 | small lengthen/red | 3.0±0.44 | 2.43±0.15 | 11.53±1.15 |
| Grăniceri 29 | red | 4.77±0.23 | 5.33±0.29 | 89.24±10.3 |
| Cruceni 249 | red | 4.4±0.1 | 5.33±0.47 | 80.22±19.87 |
| Grăniceri 25 | red | 4.23±0.21 | 5.73±1.5 | 89.24±47.7 |
| Toager 29 | red | 5.45±2.76 | 4.35±1.34 | 93.66±31.37 |
| Livezile 498 | elongated | 6.03±0.06 | 4.37±0.12 | 74.47±3.67 |
| Dolat 141 | red | 5.35±0.78 | 8.1±1.56 | 226.06±52.37 |
| Toager 44 | red | 6.1±1.07 | 8.3±0.57 | 281.43±23 |
| Livezile 498 | red | 5.17±0.55 | 7.37±0.21 | 204.88±11.88 |
| Toager 80 | red | 6.3±0.42 | 6.45±1.06 | 165.24±29.05 |
| Dolat 126 | ox heart | 6±2.83 | 8.75±3.18 | 261.86±134.92 |
| Livezile 498 | ruffled | 2.53±0.35 | 4.03±0.67 | 23.97±9.99 |
| Livezile 498 | pear | 5.34±0.82 | 3.82±0.41 | 51.51±14.21 |
| Toager 209 | round | 6.1±0.82 | 7.2±0.16 | 244.53±35.71 |
| Toager 209 | heart | 6.2±0.28 | 7±0.25 | 177.7±18.46 |
| Toager 209 | elongated | 11.3±0.79 | 4.2±0.98 | 104.25±51.04 |
| Dolat 198 | red | 4.33±0.22 | 3.6±0.34 | 37.37±9.38 |
| Livezile 498 | cherry | 3.2±0.82 | 3.23±0.68 | 23.72±11.39 |
| Cruceni 192 | red | 3.9±0.35 | 4.93±0.22 | 57.10±12.65 |
| Cruceni 102 | red | 5.8±0.72 | 7.4±0.65 | 206.2±81.71 |
| Rudna 124 | red | 4.93±0.68 | 6.93±0.95 | 146.29±48.87 |
| Giera 06 | red | 4.67±1.16 | 6.43±1.91 | 143.41±78.59 |
| Rudna 143 | small/red | 4.4±0.17 | 5.07±0.64 | 75.36±18.50 |
| Crai Nou 51 | red | 5.9±0.17 | 6.57±0.35 | 167.05±7.94 |
| Rudna 143 | red | 5.9±0.36 | 8.57±1.37 | 303.68±92.60 |
| Crai Nou 245 | red | 5.63±0.15 | 6.07±0.21 | 132.05±10.38 |
| Giera 121 | red | 4.8±0.4 | 6±0.35 | 132.38±22.04 |
| Giera 121 | elongated | 5.67±0.6 | 4.07±0.7 | 58.1±29.84 |
| Foeni 284 | red | 5±0.36 | 8.73±0.95 | 245.66±38.55 |
| Crai Nou 11 | red | 6.15±1.2 | 7.1±0.14 | 219.39±1.89 |
| Crai Nou 26 | australian red | 5.47±0.15 | 8.53±1.45 | 253.78±67.21 |
| Crai Nou 82 | red | 5.17±1.15 | 8.37±1.98 | 271.14±157.71 |
| Peciu Nou 99 | red | 6.7±0.42 | 7.85±0.21 | 240.91±12.86 |
| Uivar 305 | red | 4.03±0.25 | 7.33±1.68 | 135.40±54.2 |

| | | | | |
|---|---|---|---|---|
| Sânmartinu Sârbesc 180 | red | 4.45±0.21 | 7.15±0.92 | 155.6±61.19 |
| Sânmartinu Sârbesc 180 | big/early | 4.95±0.35 | 9.1±0.71 | 271.28±21.84 |
| Peciu nou 455 | red | 3.33±0.67 | 4.08±1.04 | 38.05±25.06 |
| Peciu Nou 708 | red | 3.55±0.92 | 4.2±0.28 | 41.73±17.66 |
| Peciu Nou 708 | red | 3.55±0.92 | 4.2±0.28 | 41.73±17.66 |
| Sânmartinu Sârbesc 411 | red | 4.25±0.35 | 5.6±0.57 | 83.97±3.05 |
| Uivar 66 | red | 7.5±0.71 | 7.35±0.92 | 256.19±43.41 |

Regarding tomatoes belonging to the red group can be observed a large genetic variability in terms of both their shape: round, elongate, ox heart, pear, wrinkled and especially in terms of their weight where values between: 425.02 g and 23.72 g were registrated.

Table 2

Experimental results regarding phenotype characteristics of tomatoes – pink group

| Local landraces | Type/Color | Height | Diameter | Weight |
|---|---|---|---|---|
| Cheglevici 165 | pink | 6.67±0.96 | 9.47±1.97 | 365.59±153.35 |
| Dolat 141 | pink | 6.25±0.64 | 9.3±1.84 | 365.43±116.78 |

After framing in the four color groups two genotypes presenting pink fruits were distinguished: Cheglevici 165 and Dolat 141. These genotypes showed phenotypic similarities in terms of height, diameter and weight of fruit.

Table 3

Experimental results regarding phenotype characteristics of tomatoes – purple group

| Local landraces | Type/Color | Height | Diameter | Weight |
|---|---|---|---|---|
| Dolat 198 | polish/purple | 5.95±0.21 | 8.6±0.57 | 377.47±18.05 |

A local landrace that presented a special feature regarding fruit color was observed in genotype Dolat 198 that showed a slight purple color. Other phenotypic traits were quite similar to tomatoes from the red group.

Table 4

Experimental results regarding phenotype characteristics of tomatoes -yellow group

| Local landraces | Type/Color | Height | Diameter | Weight |
|---|---|---|---|---|
| Cruceni 102 | yellow | 4.2±0.28 | 5.45±0.35 | 77.27±7.2 |
| Toager 29 | yellow | 4.6±0.57 | 6.15±1.2 | 117.66±61.6 |
| Livezile 498 | yellow | 6.63±1.26 | 4.25±0.68 | 80.27±32.67 |
| Dolat 244 | yellow | 5.2±0.76 | 9±0.51 | 218±86.92 |
| Crai Nou 11 | yellow | 5.9±0.71 | 5.55±0.49 | 87.86±8.13 |

Tomatoes from the yellow group presented a special feature regarding fruit color where five genotypes were framed. They had different phenotypic traits both in terms of fruit height (4.2 to 6.63 cm) diameter (4.25 to 9 cm) and weight (77.27 to 218 g).

## CONCLUSIONS

After collecting the Timis local landraces it can be observed that there is a strong genetic variability manifested by specific color of the fruit, which were then framed in four color groups: red, purple, pink and yellow

A strong variability was observed regarding biometric characters was observed in tomatoes red group where significant differences were found mainly in terms of fruit weight.

## ACKNOWLEDGEMENT

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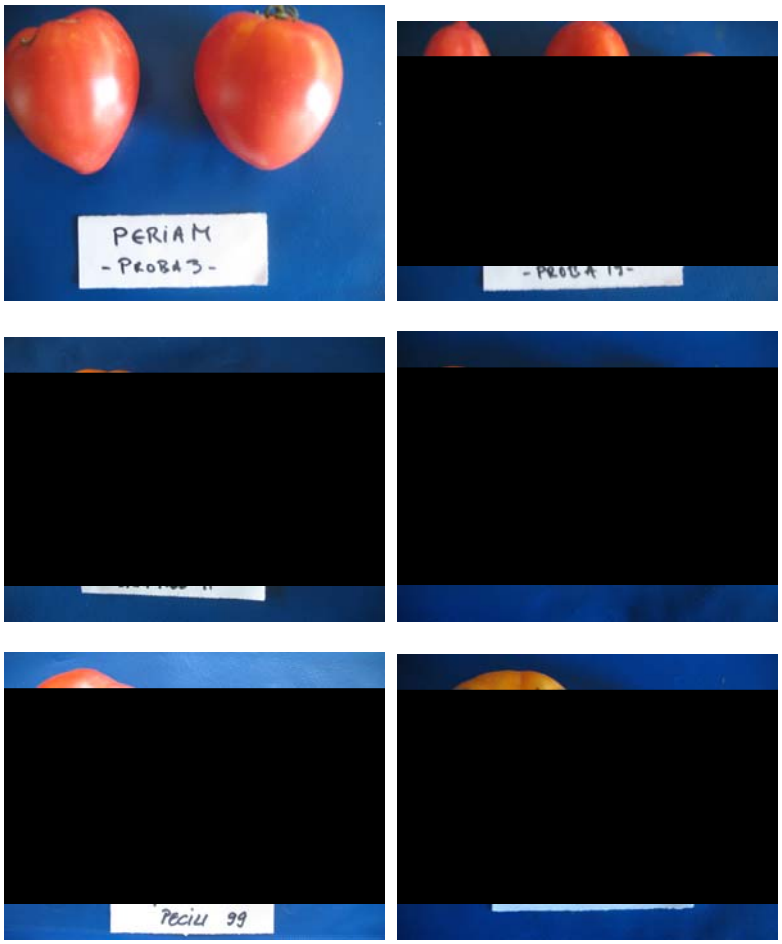
* www.whfoods.com

* http:// news.bbc.co.uk

Annex 1

Aspects of some tomato genotypes



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# THE STUDY OF AIR TEMPERATURE IN THE CÂMPULUNG MUSCEL DEPRESSION

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***Key words:*** *temperature, temperature deviation, thermal threshold, extremes*

## ABSTRACT

*Over the past 50 years, the mean annual air temperature in the Câmpulung Muscel Depression shows an increasing trend of about 1ºC, from 7.9 ºC to 8.9 ºC, from 1961-1990-reference period. The largest increase in the thermal values were recorded in the decade 2001-2010, when the mean annual temperature was 9.0 ºC, compared with 7.9 ºC, specific for 1961-1990-reference interval..*

*The absolute minimum temperature recorded at Câmpulung was -23.7°C (January, 23 1963), and the absolute maximum was 35.8°C (July 4, 2000). Frost days typically occurred in October. The last frosts occurred in April, but often they can also occur in May, with 3-5 events per decade. Located at the foot of the Iezer Mountains, the Câmpulung Depression, is kept free from the cold continental air from the East and North-East.*

## INTRODUCTION

Today, more than ever, the interest for weather phenomena and their impact on human and economic activity is increasingly obvious. Agriculture, in all its segments, is directly affected by these phenomena and their effects, either positive or negative, cannot be downplayed or ignored (Enache, 2010; Nikolova & Mochurova, 2012).

Agricultural production varies from one year to another, being significantly influenced by fluctuations in the climatic conditions and especially by the production of extreme weather events (Anav & Mariotti, 2011; Eitzinger et al., 2008).

The in-depth study of the climatic factors on a certain area establishes measures of defense against the climatic accidents and severe weather phenomena, such as drought, extreme temperatures and strong winds (Ramoset al., 2011; Zurbenko & Cyr, 2011; Sandu et al. 2010).

At present, after the fragmentation of agricultural areas, the situation is more disadvantageous in the Câmpulung Muscel Depression, an area of long tradition in breeding and cultivation of the last surface of soil. The areas cultivated, the structure of crops and yields have recorded significant changes after 1990 (Statistical Yearbook of Romania).

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For these reasons, the proposed study aims to collect data on the climatic parameters with a view to optimising agricultural production in the Câmpulung Muscel Depression, as there are no other studies for this area.

## MATERIALS AND METHODS

For this study we have used the daily climatic data archive from the Câmpulung meteorological station for the interval 1961-2010 (N.M.A. Bucharest):

- mean, minimum and maximum daily, monthly, annual, decadale and tridecadale temperatures;
- linear and polynomial trend;
- sliding means, calculated on 5-year intervals;
- assessment of monthly and annual heat according to the Hellmann criterion;
- quantitative analysis over certain thresholds.

## RESULTS AND DISCUSSIONS

The analysis and interpretation of the climatic data concerning temperature presents a great importance, both for the theoretical framing in a climatic zone and especially for practical reasons.The types and stages of vegetation, as well as the entire human activity are related to the constancy of each various stage of the annual air temperature cycle (months, seasons, semesters).

At the Câmpulung Meteorological Station, the mean annual air temperature of the 1961 - 1990 reference interval was 7.9°C. In the past 50 years, the mean air temperature was 8.2°C, with a growing trend of about 1°C, to 7.9°C to 8.9°C, compared to the 1961-1990-reference interval (fig. 1).

The evolution curve, plotted for sliding means, highlighted a colder period, starting with the 1967-1971 slide interval until the 1981-1985 slide interval. Starting with the 1982-1986 slide interval, a steep increase was observed in the trend values of the sliders, which persisted until the 2004-2008 slide interval, after which it begans to fall (fig.2).

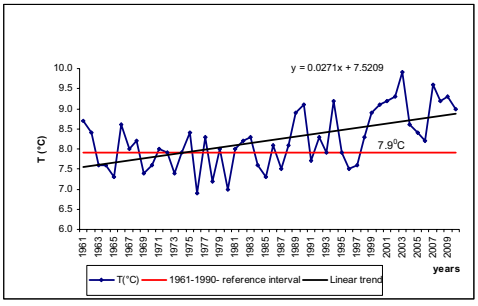


Fig. 1. Mean annual temperature, recorded at Câmpulung meteorological station (1961-2010)

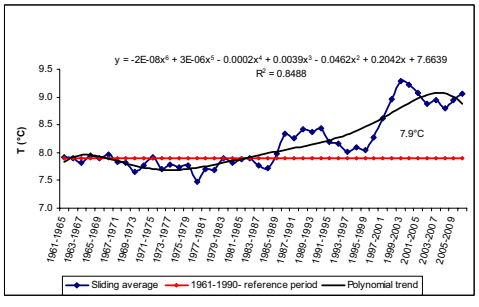


Fig. 2. Sliding means temperature and their trend at Câmpulung meteorological station (1961-2010)

According to the Hellmann criterion, in the 50 years under study, 20% were considered warmer years, 10% warm years, 10% were cold, the remaining 60% being considered normal from the heat point of view (fig. 3).

For agriculture, it is necessary to take into account the evolution of temperatures during thetypical months of January, April, July and October (N.M.A. Bucharest, 2008).

The mean temperature of January in the 1961-1990 reference interval, recorded at the Câmpulungmeteorological station, was -2.5°C (fig. 4). The lowest mean of January was - 8.4°C, in 1963, and the highest was 2.6 °C, in 2007.

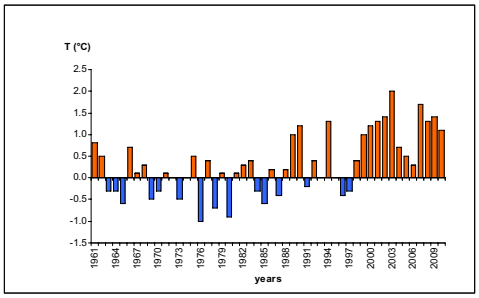


Fig. 3. Annual mean temperature deviations from 1961-1990 reference interval, recorded at Câmpulung meteorological station (1961-2010)

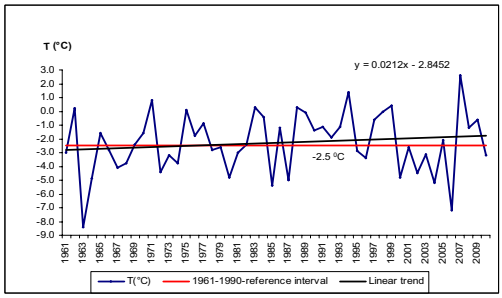


Fig. 4. January month mean temperature, recorded at Câmpulung meteorological station (1961-2010)

The mean temperature deviation of January, in the period 1961-2010, recorded values between -5.9°C in 1963 and + 5.1°C in 2007, compared to 1961-1990- reference interval, at a predominant positive ratio of 52% (fig. 5).

Knowing the thermal regime in spring, the month of April, presents a great importance for agriculture, because it indicates the beginning of the vegetation season for most cropson the one hand, and the development of many agricultural workson the other hand.

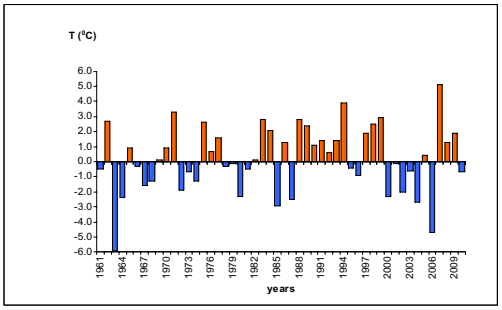


Fig. 5. January mean temperature deviations, recorded at Câmpulung (1961-2010)

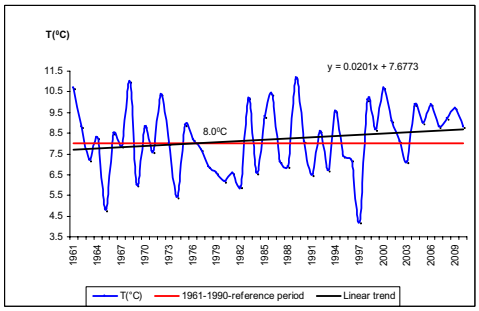


Fig. 6. April mean temperature, recorded at Câmpulung meteorological station (1961-2010)

The 1961-1990 reference interval for April was 8.0°C. The mean temperature this month showed large variations from one year to another, from 4.2°C in 1997 to11.2°C in 1989, with a rising trend of about 0.7°C over the 1961-1990- reference period (fig. 6).

The mean temperature of July presents a growing trend of about 2.4°C compared to the 1961-1990- reference period, i.e.17.7°C (fig. 7). The lowest temperature was 15.8°C in 1979, and the highest was 22.7°C, in 2002.

The July mean temperature deviations recorded values between -1.9°C in 1979 and + 5.0°C in 2002, with apredominant positive ratio of 64%; these were grouped in the 1987-2010range, except for the years 1993 and 1997 (fig. 8).

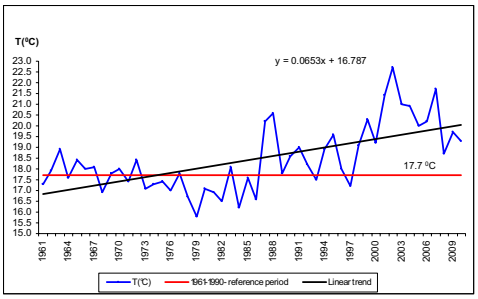


Fig 7. July mean temperature, recorded at Câmpulung meteorological station (1961-2010)

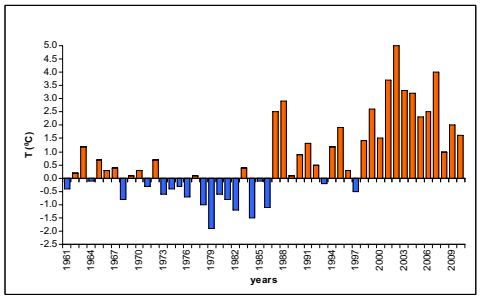


Fig. 8. July mean temperature deviations, recorded at Câmpulung meteorological station (1961-2010)

In the Câmpulung Depression, the first day of the annual interval with the daily mean positive temperatures, according to the histogram (fig. 9), was recorded on February 21, and the last day on December 9, with a duration of 292 days, the annual amount of positive temperatures of 3,285.3°C (fig. 10).

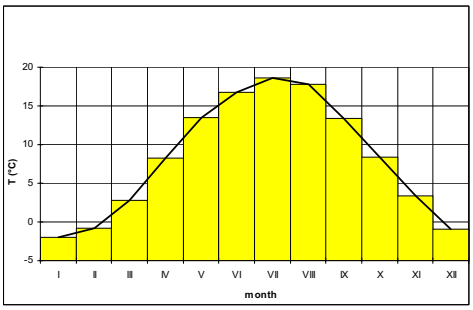


Fig. 9. Mean monthly temperature histogram, at Câmpulung weather station (1971-2010)

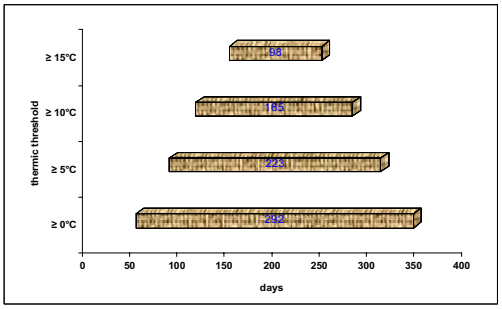


Fig. 10. Mean duration of characteristic temperature interval at Câmpulung (1971-2010)

Extreme temperatures constitute distinct parameters, different hourly means the largest and the smallest; they are instantaneous values and occur at a time and between the observation terms (N.M.A. Bucharest, 2008).

The absolute minimum temperature recorded at Câmpulung was -23.7°C (January 23, 1963), and the absolute maximum was 35.8°C (July 4, 2000). The largest absolute amplitudes of extreme temperatures were recorded during the cold season and had values between 39.9°C in December and 42.3°C in March (fig. 11).

Several features of the evolution of annual temperatures overlap their monthly features evolution; however, unlike the latter, the daily temperatures provide the opportunity to detail some of these features.

At Câmpulung, the lowest temperatures were recorded in the latter part of January. In 2010, between 20-28 January, the absolute minimum temperatures ranged between -10.3°C and -19.5°C, while the mean temperature varied between-8.0 °C and-14.8°C (fig. 12). The highest values of temperature, with the maximum over 30.0°C, were recorded in the second decade of August. Maximum temperatures of 29.0-30.0°C were also recorded in the second decade of June and July.

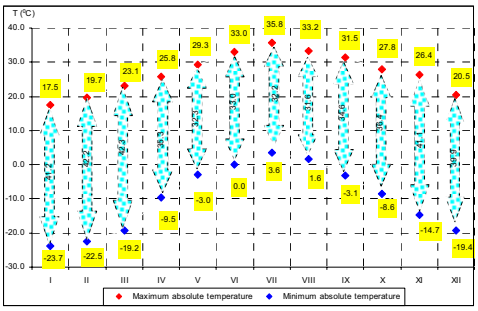


Fig. 11. Amplitude of extreme absolute temperatures, recorded at Câmpulung (1961-2010)

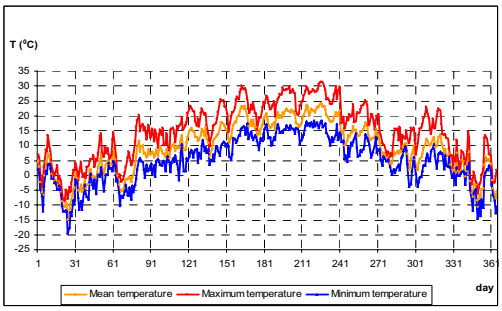


Fig. 12. Evolution of daily mean, maximum and minimum temperatures, at Câmpulung in 2010

The falling of air temperature below 0°C in spring and autumn occurred episodically and caused significant damage for agriculture.

The probability of last frost in air occurring in spring was 27% after 1 May, 15% after 5 May, and 5% after 10 May (fig. 13).

The probability of the first frost in autumn was about 8% before 1 October, 20% until 5 October, and 32% until 10 October (fig. 14).

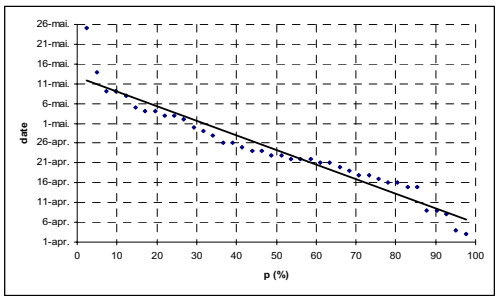


Fig. 13. Assurance curve of the last frost in air, at Câmpulung (1971-2010)

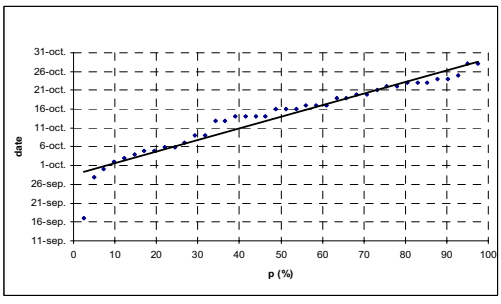


Fig. 14. Assurance curve of the first frost in air, at Câmpulung (1971-2010)

Situated at the foot of the Iezer Mountains, the Câmpulung Muscel Depression is kept free from the cold continental air coming from the East and North-East.

## CONCLUSIONS

Over the past 50 years, the mean air temperaturein the Câmpulung Muscel Depression was 8.2°C. It presented an increasing trend of about 1°C, to 7.9°C to 8.9°C, compared to the 1961-1990- reference period, ranging between 6.9°C (1976) and 9.9°C (2003).

According to the Hellmann criterion, during the 50 years under study, 20% were considered warmer years, 10% warm years, 10% were cold, the remaining 60% being considered normal from the heat point of view.

In the Câmpulung Depression, the first day of the annual interval with daily mean positive temperatureswas recorded on February 21, and the last day on December 9, with a duration of 292 days, and the annual amount of positive temperatures of 3,285.3°C.

The absolute minimum temperature recorded at Câmpulung was -23.7°C (January 23, 1963), and the absolute maximum was 35.8 °C (July 4, 2000).

The probability of last frost in air occurring in spring was 27% after 1 May, 15% after 5 May, and 5% after 10 May, the probability of the first frost in autumn was about 8% before 1 October, 20% until 5 October, and 32% until 10 October.

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**Vol. XVII ( LIII ) - 2012**

# ASSESSMENT OF THE TOXICITY OF SOME PHYTOPHARMACEUTICAL PRODUCTS FROM *CAPRIFOLIACEAE* FAMILY BY *TRITICUM* BIOASSAY

Chirigiu L.[1], Bubulică Maria Viorica[1], Averis Larisa-Marina-Elisabeth[1]

***Keywords*****:** *Triticum test, phytopharmaceutical products, genotoxic potential, cytotoxicity*

## ABSTRACT

*The aim of this research was to accomplish a comparative study of inhibitory effect of Sambucus ebulus, Viburnum opulus şi Lonicera tatarica aqueous extracts on root elongation of wheat caryopsis (Triticum vulgare). Varying concentrations of the same extract were tested between 0.25 and 2 %. Triticum test revealed that the extracts have a stimulating effect on cell division at low concentrations (0,25%), while at high concentrations (1-2%), the effect is reversed, mitoinhibitory. We found a high potential of wheat caryopsis germination activation for Se2, Lt2 and Vo2 extracts at 0,25 % concentration, especially in the first 3-4 days of observation.*

## INTRODUCTION

*Viburnum opulus* species is used for medicinal purposes (2% infusion, decoction or hydroalcoholic extracts) in the treatment of stomach or uterine bleeding and hemorrhoids (Bown, 1995).

*Lonicera tatarica* is a species of Asian origin used as an infusion to treat coughs, asthma and detoxify the body (flower buds) (Chevallier, 1996). The Lonicera species are frequent in the flora of our country and can represent cheap therapeutic alternatives, easily accessible and without side effects.

*Sambucus ebulus is a species of European-Asian origin, known in the popular medicine. Iits roots (Ebuli radix) areused especially for the analgesic, bacteriostatic and diuretic action* (Woods, 1993).

Phytobiological assay of plant extract provide information on their cytotoxicity. The most common approach for this purpose is *Triticum* bioassay of dr. Gr. Constantinescu, along with other tests performed on roots of *Allium sativum* and *Vicia faba* (Yi and Meng , 2003).

## MATERIALS AND METHODS

The influence of some aqueous extracts obtained from Viburnum opulus, *Sambucus ebulus* and *Lonicera tatarica* on the germination, growing and mitosis film of *Triticum vulgare* was traced. In order to evaluate this influence, we used Constantinescu method, *Triticum* bioassay (Ancuceanu et al., 2005; Constantinescu et al., 1963).

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The experiment took place from May 20 to 25, 2012, on wheat caryopsis (*Triticum vulgare, Dropia* variety*,* Gramineae), uniformly selected.

These were placed for 24 hours in a container and were moistened with distilled water. During the five days of experimentation, in the room where the experiment took place was kept a constant temperature and illumination.

Every 5 caryopsis, with the main root of at least 0.5 – 1 cm length were placed in Petri dishes.

*Plant material*

The plant material was represented by fruits and bark of *Viburnum opulus,* leaves and fruits of *Sambucus ebulus* and fruits and flowers of *Lonicera tatarica*. The raw material was dried in a dark room and was pulverized in a stainless grinder.

Aqueous extracts were prepared with concentrations of 2, 1, 0.5 and 0.25%. A control sample was prepared using distilled water (Bucur et al., 2005).

Assessment of length elongation was performed using linear measurement technique, from the initial moment of treatment every 24 hours during 5 consecutive days, this period representing the most active growth phase.

Morphological changes (root and epicotyl aspect and length) were observed.

Inhibition was calculated by the formula:

$$I(\%) = \frac{L_M - L_p}{L_M} \cdot 100$$

where:

$L_M$ = average root length of the blank;

$L_P$ = average root length of the sample;

For microscopic study, main roots were sectioned at a distance of about 5 mm of the top, plant material was then fixed for approximately one hour in 2 mL 45% acetic acid solution.After removing the fixing solution, plant material was hydrolyzed with hot HCl 1N (on water bath at 60 °C for 5 minutes). After removal of hydrochloric acid plant material was colored with basic fuxin.

## RESULTS AND DISCUSSION

Evaluation of extracts cytotoxicity on *Triticum vulgare* caryopsis was assessed by measurements of root elongation. Varying concentrations of the same extract were tested between 0.25 and 2%.

Within 48 hours, germination evolution could indicate an influence of the extract on the enzymatic systems characteristic to processes (Ioniță and Dinu, 2004).

After the test period, a bisens influence of the extracts on root elongation was observed. Thus, it was found that at low concentrations, in the first few days of the experiment the extracts have a stimulating effect on cell division (figure 1), while at high concentrations the inhibition is proportional with concentration at all samples (mitoinhibitory action). At $S_{e1}$ there was a very strong inhibition by comparison with $S_{e2}$ to which is observed in the first four days a slight enhancement of primary root growth compared with the control sample.

At the end of the 120 hours of testing, we found that inhibition was over 80% for extractive solutions $S_{e1}$ (a – 2 %,b-1%,c – 0.5 %), $V_{o2}$ (a – 2 %,b – 1 %) and $L_{t2}$ (a – 2 %). Aqueous extracts with concentrations between 0.25 to 1% of $L_{t1}$ have a moderate mitoinhibitory effect (figure 1), epycotil maintaining green throughout the study. We noted that for all samples the appearance of the root has changed, become thicker and forked.

An interesting phenomenon was noted at the aqueous extract of fruits of *V. opulus* that for concentrations of 1 to 2% favored the development of *Mucor* species, probably due to fructosis contained in the extract. Also, in these samples rootlet become brown and solution became opalescent.

In terms of mycotic film changes, a chromatoclastic effect (elongated, hypertrophied nucleus, dispersed nucleoli and chromosomes) was observed for 2 % concentration of $S_{e1}$, $V_{o1}$ and $V_{o2}$ extracts.

Flavonoids and the presence of other classes of secondary metabolites (for example saponosids) in this type of extracts could be responsible for this effect. The moderate genetic clastogenic (genotoxic) effect remains worthy of consideration.

In the case of the other solutions no such effect was observed, all stages of cell division resulting normal. However, a heterocromatinization of the genetic material in prophase was observed, along the nuclear envelope.

We can appreciate that at low concentration, the cellular responses allow assessments of the possible applications in the biomedical field.

## CONCLUSIONS

*Triticum* test showed a pronounced mitoinhibitory effect just at high concentration (1 – 2%). We found a high potential for activation of wheat caryopsis germination at 0,25 % concentration, especially in the first 3-4 days of observation, but with no statistically significance. The clastogenic (genotoxic) effect remains worthy of consideration for $S_{e1}$, $V_{o1}$ and $V_{o2}$. This fact indicates certainly cytotoxic and cytostatic possible action of this two type of extracts. The cellular responses indicate possible applications in the biomedical field or plant physiology field.

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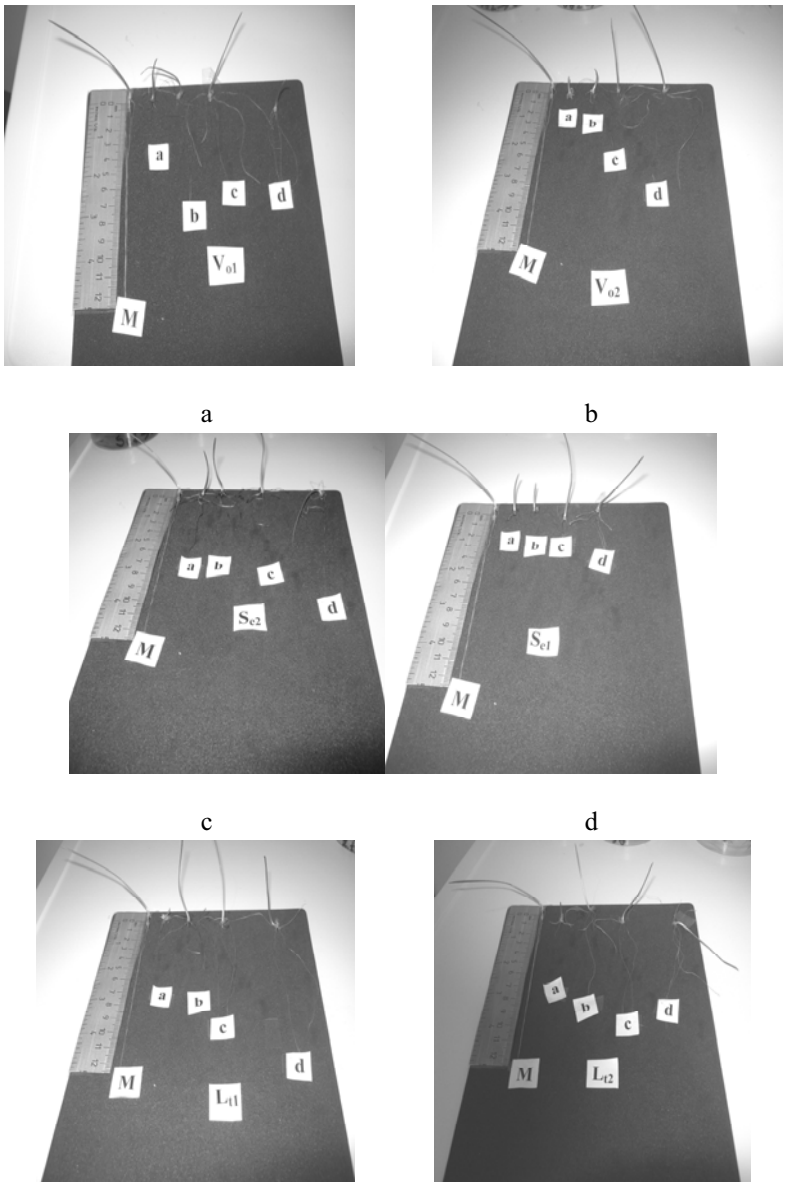


Figure 1. Root elongation after 120 hours (a – 2% solution, b –1 % solution, c – 0,5 % solution, d –0,25 % solution): a – *Viburnum opulus* bark ($V_{o1}$); b - *V. opulus* fruits ($V_{o2}$); c - *Sambucus ebulus* leaves ($S_{e1}$), d - *S. ebulus* fruits ($S_{e2}$), e – *Lonicera tatarica* flowers ($L_{t1}$), f - *L. tatarica* fruits ($L_{t2}$).

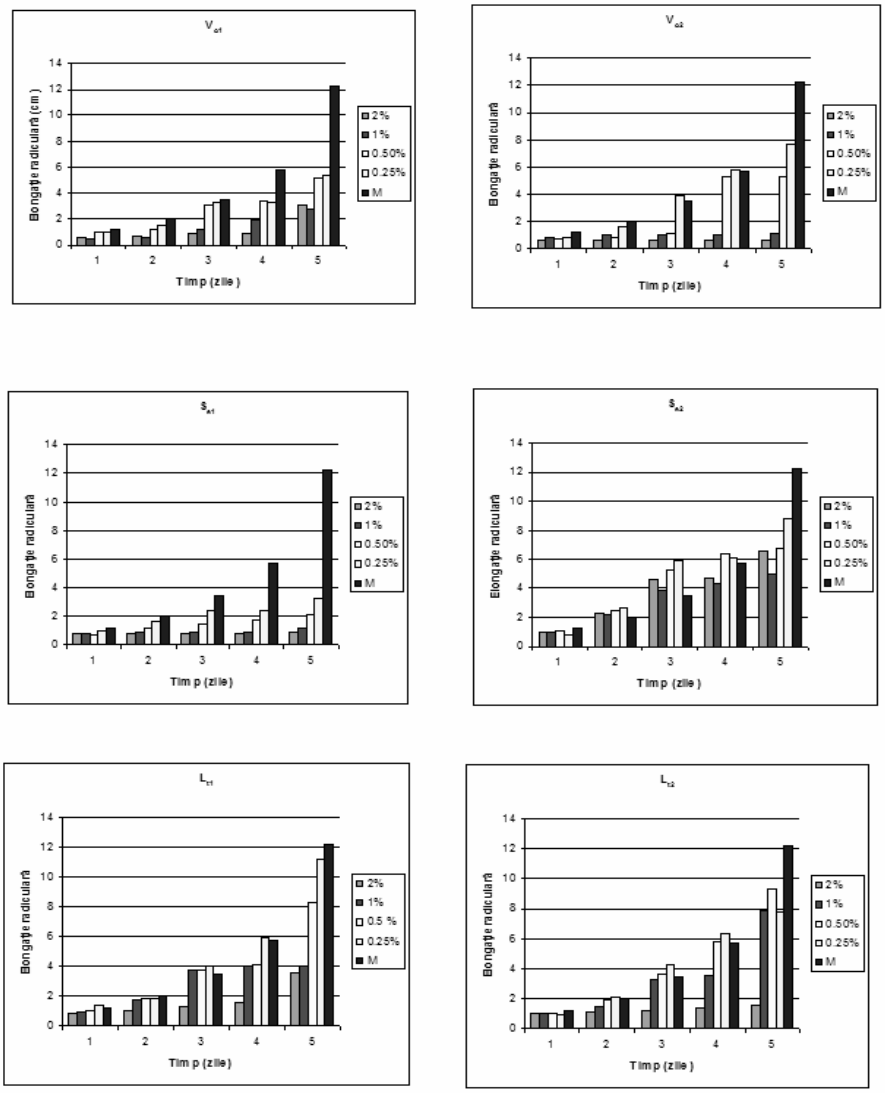


Figure 2. Root elongation during the five days of experiment. Series 1 – 2% solution, series 2 – 1 % solution, series 3 – 0,5 % solution, series 4 – 0,25 % solution, $V_{o1}$ – *Viburnum opulus* bark; $V_{o2}$ – *V. opulus* fruits; $S_{e1}$ – *Sambucus ebulus* leaves, $S_{e2}$ – *S. ebulus* fruits, $L_{t1}$ – *Lonicera tatarica* flowers, $L_{t2}$ – *L. tatarica* fruits.

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# THE DYNAMIC OF THE ATTACK EVOLUTION OF THE PATHOGENS VENTURIA INAEQUALIS (CKE.) WINT. AND PODOSPHAERA LEUCOTRICHIA (ELL. et EVERH.) SALM AND IN APPLE TREE CULTURE FROM THE TEACHING RESORT BANU MARACINE BETWEEN 2010-2012

Ciciu Tudosie Roxana Elena[1], Mitrea Rodi[2*]

***Keywords***: *apple tree, pathogen, evolution, attack, dynamic*

## ABSTRACT

*The research regarding the dynamic of the attack evolution of pathogens Venturia inaequalis and Podosphaera leucotricha in the apple culture from the Teaching Resort Banu Maracine Craiova of 19 varieties of apple trees grown pointed out that these showed a great variability during the three years in which they were studied.*

*The 19 varieties behaved differently to the attack of the two pathogens, different degrees of attack being recorded, varying degrees of attack depending on climatic conditions but also on apple phenology.*

## INTRODUCTION

The powdery mildew produced by the fungus *Podosphaera leucotrichia (Ell. et Everh.) Salm* and apple scab produced by the fungus *Venturia inaequalis (Cke.) Wint* are the main diseases of the apple tree, with major economic implications, both because of very favorable weather conditions for these pathogens and sensitivity of varieties existing in culture.

Many researchers were concerned about the biology and ecology of fungi *Podosphaera leucotricha (Ell. et Everh.) Salm* and *Venturia inaequalis (Cke.) Wint*, including: MacHardy E.W.1996, MacHardy E.W.,Gadoury M.D. Gessler C. 2001.Gessler C.,Patocchi A.,Sansavini S.,Tartarini S.,Gianfranceschi L.,2006;Kranz,J.2008;in Romania data about the behavior of some apple varieties to the attack of these fungi are presented by: Savescu A et al.1951; Rădulescu andMozes,1959; Rădulescu, 1972; Tomsa M,Tomsa E 2003, Stratu V., 2006**;** Timar A.2009; Sestras R. 2011.The climatic conditions create the environment in which the agricultural biocenosis develop and influence directly the evolutionary cycle of pathogen.

Every climatic factor has a precise determined influence on both host plant and on specific damaging agents. Thus, knowledge of host plant phenology, biological reserve of the pathogen and climatic conditions is the one that highlights the evolutionary dynamics of pathogens according to which in practice a correct warning can be made of the treatments

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to prevent and combat.Sporulation, germination, growth and development, mode of infection, life cycle and evolution of pathogens and plant resistance to fungal infections are influenced by external environmental factors.

Temperature and rainfall are factors whose action directly impacts the performance dynamics of infection.

Temperatures too low as well as too high ones, are limiting factors for life cycle of fungi, each having an optimal temperature that influences the dynamics of attack evolution during the vegetation period.

Along with temperature, rainfall is a limiting or contributory factor, considered together, the temperature and the humidity are the so-called hydrothermal predisposition factor of great importance in creating a state of predisposition of plants to infection with various fungi, for this one being calculated a hydrothermal predisposition index. (Tr. Savulescu).It is known the fact that in order to reduce crop losses, rational measures of prophylaxis and treatment must be applied, according to pathogen biology, environmental conditions, as well as the degree of resistance of varieties, in this paper we present the dynamics and evolution of pathogens in correlation with the changing climate and host plant phenophases development.

## MATERIALS AND METHODS

In order to establish the dynamic of attack evolution of the two species of fungi, macroscopic observations were made in the apple culture from The Teaching Resort Banu Maracine Craiova depending on the phenophase of vegetation that were correlated with the changing climate.The biology of fungus was observed in the vegetation period, during the years 2010 – 2012, being correlated with climatic conditions: temperature, humidity, precipitation depending on which the warnings of phytosanitary treatments were made.

The attack appreciation made by the two pathogens in the three years of research resulted in scoring according to the existing methodology of frequency (F%) and intensity (I%) on 5 trees / variety of 200 leaves per tree, and the degree of attack (DA%) was calculated in the laboratory.

## RESULTS AND DISCUSSIONS

Fungus Venturia inaequalis winters in the peritechia form in fallen leaves, under stromatic mycelium form or resistance mycelium in bud scales.The disease is favored by heavy rainfall or atmosphere saturated with water vapor.

Primary infections produced by ascospores occur after 37 -73 hours after the moment of the design of ascospores from asci at the temperature of 5 ° C, and the incubation period is 22 days; at a temperature of 18 ° C the infection by ascospores takes 9-18 days, and the incubation period is 8 days.

Secondary infections by conidiums occur if apple organs are wetted for a period of 4-18 hours and temperatures between 15-25 ° C (optimum 19-20 ° C).For this pathogen The Weather Station and Warning of Phytosanitary Directorate Dolj warned about the date and time of making the treatments (Table 1).

From the data concerning the number of treatments and warning time, it emerges the fact that in the period under study, the minimum number of treatments was 7 in 2011, in different phenophases, starting with the spreading flower and corolla fluffiness until early July.In terms of evolutionary dynamic scab attack, DA was recorded for each variety during the three years, as shown in table 2.

The variety Goldenspur registered the highest value of the DA of 53.35% in 2010, Elton 10.57% in 2011 and Arlet 9.47% in 2012.The varieties with the lowest DA were: Early Red 0.74% in 2010, Elstar 1.74% in 2011 and Ionagold 2.30% in 2012.

The variety with the highest average of DA during the 3 years was Goldenspur 20.76%, the lowest average being recorded by the variety Elstar with 2.26%.The varieties Florina, Sure Prize, Generos, Prima, Akane and Arkcham did not show any apple scab attack during the three years, behaving as immune.The year with the strongest attack and the largest number of attacked leaves in all varieties was 2010, also registering an increased number of treatments.

Table 1

Number and date of treatments application in apple culture in The Teaching Resort Banu Maracine Craiova for the pathogen *Venturia inaequalis* (Cke.) Wint.during the period between 2010-2012

| Year | Number of treatments warned | Warning date | Optimal period of treatment effect |
|---|---|---|---|
| 2010 | 1. | 07.04. | spreading of the inflorescence and corollafluffiness |
| | 2. | 23.04 | 15-20 % flowers with petals shaken |
| | 3. | 12.05 | 14 May-18 May |
| | 4. | 20.05 | 24 May - 28 May |
| | 5. | 28.05 | 01-05 June |
| | 6. | 17.06 | 21-25 June |
| | 7. | 25.06 | 01-05 June |
| | 8. | 15.07 | 20-24 June |
| 2011 | 1. | 05.04 | spreading of the inflorescence and corollafluffiness |
| | 2. | 26.04 | 15-20 % flowers with shaken petals |
| | 3. | 19.05 | 21 May - 25 May |
| | 4. | 25.05 | 31 May– 4 June |
| | 5. | 04.07 | 08 -12 July |
| | 6. | 18.07 | 31 July- 04 August |
| | 7. | 23.06 | 28 June – 02 July |
| 2012 | 1. | 09.04 | spreading of the inflorescence and corollafluffiness |
| | 2. | 17.04 | 15-20 % flowers with shaken petals |
| | 3. | 11.05 | 14 May-18 May |
| | 4. | 17.05 | 24 May - 28 May |
| | 5. | 25.05 | 10-14 June |
| | 6. | 14.06 | 20 June – 24 June |
| | 7. | 25.06 | 30 June – 04 July |
| | 8. | 05.07 | 20 -24 July |

The variety with the highest average of DA during the 3 years was Goldenspur 20.76%, the lowest average being recorded by the variety Elstar with 2.26%. The varieties Florina, Sure Prize, Generos, Prima, Akane and Arkcham did not show any apple scab attack during the three years, behaving as immune.

The year with the strongest attack and the largest number of attacked leaves in all varieties was 2010, also registering an increased number of treatments.

Regarding the fungus *Podosphaera leucotricha,* it winters under the form of resistance mycelium in vegetative buds and flowery buds. In spring contaminated buds give sprouts, respectively infected flowers.

These represent the first outbreaks of primary infections during flowering, on the surface of the attacked bodies developing conidiums, which re-contaminate the vegetative and flowery buds, ensuring fungus propagation and transmission of the disease by resistance mycelium that winters inside the buds.

The temperature during the winter acts directly on the resistance mycelium of the fungus, the lower it is the more destroyed is the mycelium and the disease development is inhibited the following year.

During the vegetation period the fungus spreads through conidiums, their germination and haustoria penetration in the epidermal cells being achieved at temperatures between 19-20° C and a relative air humidity of 90%.

In the climatic conditions of the year 2010 the treatments to fight against *Podosphaera leucotrichia* were warned in the following phenological phases: when 10-15% of the buds have burgeoned, when the inflorescence spread and the corolla fluffed, then when the flowers started to shake their petals; then two treatments in May, June and July.

Table 2

The dynamic of the attack evolution of pathogens *Venturia inaequalis (Cke.) Wint* in the different variety of apple tree in apple culture from The Teaching Resort Banu Maracine Craiova during 2010-2012

| Crt no. | Studied apple tree varieties | 2010 | | | 2011 | | | 2012 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of analysed/attacked leaves | | DA % | Number of analysed/attacked leaves | | DA% | Number of analysed/attacked leaves | | DA% |
| 1 | Florina | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 2 | Idared | 1000 | 450 | 11,57 | 1000 | 0 | 0 | 1000 | 220 | 5,26 |
| 3 | Starkrimston | 1000 | 280 | 1,89 | 1000 | 400 | 6,28 | 1000 | 345 | 8,24 |
| 4 | Goldenspur | 1000 | 800 | 53,35 | 1000 | 200 | 2,46 | 1000 | 290 | 6,46 |
| 5 | Ionagold | 1000 | 325 | 3,98 | 1000 | 150 | 1,89 | 1000 | 180 | 2,30 |
| 6 | Ionathan | 1000 | 250 | 2,49 | 1000 | 200 | 4,32 | 1000 | 430 | 6,35 |
| 7 | Elstar | 1000 | 345 | 2,07 | 1000 | 210 | 1,74 | 1000 | 315 | 2,96 |
| 8 | Sure Prise | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 9 | Generos | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 10 | Elton | 1000 | 575 | 11,92 | 1000 | 490 | 10,57 | 1000 | 490 | 8,25 |
| 11 | Royal | 1000 | 850 | 13,92 | 1000 | 675 | 6,17 | 1000 | 565 | 7,82 |
| 12 | Arlet | 1000 | 800 | 35,72 | 1000 | 550 | 8,12 | 1000 | 650 | 9,47 |
| 13 | Elista | 1000 | 150 | 0,88 | 1000 | 445 | 5,19 | 1000 | 580 | 4,85 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Early Red | 1000 | 200 | 0,74 | 1000 | 850 | 24,64 | 1000 | 320 | 5,49 |
| 15 | Fuji | 1000 | 445 | 12,38 | 1000 | 485 | 8,23 | 1000 | 385 | 6,33 |
| 16 | Prima | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 17 | Akane | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 18 | Arkcharm | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 19 | Granny mith | 1000 | 375 | 4,08 | 1000 | 220 | 6,21 | 1000 | 240 | 4,52 |
| | Media | 1000 | 307,6 | 8,16 | 1000 | 256,6 | 4,52 | 1000 | 263,7 | 4,43 |

Under the studied period the lowest number of treatments was 8 and they were recorded in 2011, the climatic conditions imposed treatments being applied until the end of July-the beginning of August, as shown in the data from table no. 3.

Table 3

Number and application date of treatments in apple culture from The Teaching Resort Banu Maracine Craiova for the pathogen *Podosphaera leucotrichia* (Ell. et Everh.) during the period 2010-2012

| Year | Number of treatments warned | Warning date | Optimal period of treatment effect |
|---|---|---|---|
| 2010 | 1. | 26.03. | when 10-15% from buds have burgeoned |
| | 2. | 07.04 | spreading of the inflorescence and corolla fluffiness |
| | 3. | 23.04 | 15-20 % flowers with shaken petals |
| | 4. | 12.05 | 14 May -18 May |
| | 5. | 20.05 | 24 May - 28 May |
| | 6. | 28.05 | 01-05 June |
| | 7. | 17.06 | 21-25 June |
| | 8. | 25.06 | 01-05 July |
| | 9. | 15.07 | 20-24 July |
| 2011 | 1 | 24.03 | when 10-15% from the buds have burgeoned |
| | 2. | 05.04 | spreading of the inflorescence and corolla fluffiness |
| | 3. | 26.04 | 15-20 % flowers with shaken petals |
| | 4. | 19.05 | 21 May - 25 May |
| | 5. | 25.05 | 31 May – 4 June |
| | 6. | 04.07 | 08 -12 July |
| | 7. | 18.07 | 31 July- 04 August |
| | 8. | 23.06 | 28 June – 02 July |
| 2012 | 1. | 26.03 | when 10-15% from the buds have burgeoned |
| | 2. | 09.04 | spreading of the inflorescence and corolla fluffiness |
| | 3. | 17.04 | 15-20 % flowers with shaken petals |
| | 4. | 11.05 | 14 May -18 May |
| | 5. | 17.05 | 24 May - 28 May |
| | 6. | 25.05 | 10-14 June |
| | 7. | 14.06 | 20 June – 24 June |
| | 8. | 25.06 | 30 June – 04 July |
| | 9. | 05.07 | 20 -24 July |

In terms of evolutionary dynamic of powdery mildew attack, DA was recorded for each variety during the three years, as shown in table 4.In terms of evolutionary dynamic of mildew attack, DA was recorded for each variety during the three years, as shown in table No. 4.The varieties with the highest DA were: Goldenspur 11.82% in 2010, Idared 23.15% in 2011 and Ionathan 12.93% in 2012.The variety with the lowest DA during the 3 years was Generos - 1% in 2010, 1.67% in 2011 and 2.35% in 2012.The variety with the highest average of DA during the 3 years was Idared, the lowest recorded average being recorded by Generos variety.

The varieties Florina, Starkrimston, Elstar, Royal, Arlet, Elista, Early Red, Fuji, Prima, Arkcham and Granny Smith did not show any attack of powdery mildew on the leaves during the three years, behaving as immune.

Table 4

The dynamic of the attack evolution produced by pathogen *Podosphaera leucotrichia* (Ell. et Everh.) in different varieties of apple tree in apple culture from The Teaching Resort Banu Maracine Craiova during 2010-2012

| No. crt. | Studied apple varieties | 2010 | | | 2011 | | | 2012 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 19 | Granny mith | Number of analysed leaves | attacked | DA % | Number of analysed leaves | attack | DA% | Number of analysed leaves | attack | DA% |
| | Media | 1000 | 132,4 | 2,31 | 1000 | 185 | 4,11 | 1000 | 156,3 | 2,69 |
| 1 | Florina | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 2 | Idared | 1000 | 500 | 7,5 | 1000 | 750 | 23,15 | 1000 | 450 | 10,5 |
| 3 | Starkrimston | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 4 | Goldenspur | 1000 | 275 | 11,82 | 1000 | 375 | 12 | 1000 | 570 | 10 |
| 5 | Ionagold | 1000 | 200 | 4,2 | 1000 | 270 | 5,76 | 1000 | 260 | 5,30 |
| 6 | Ionathan | 1000 | 625 | 7,81 | 1000 | 825 | 18,63 | 1000 | 730 | 12,36 |
| 7 | Elstar | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 8 | Sure Prise | 1000 | 280 | 3,67 | 1000 | 380 | 3,08 | 1000 | 240 | 2,69 |
| 9 | Generos | 1000 | 150 | 1 | 1000 | 190 | 1,67 | 1000 | 210 | 2,35 |
| 10 | Elton | 1000 | 300 | 6 | 1000 | 490 | 10,57 | 1000 | 330 | 5,25 |
| 11 | Royal | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 12 | Arlet | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 13 | Elista | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 14 | Early Red | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 15 | Fuji | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |
| 16 | Prima | 1000 | 0 | 0 | 1000 | 0 | 0 | 1000 | 0 | 0 |

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# GEOPARK PLATEAU MEHEDINȚI, A LITTLE KNOWN BOTANICAL EDEN, NESTLED IN THE SOUTH CARPATHIANS (ROMANIA)

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***Keywords***: *flora*, *endemites*, *rare plants*, *serpentine*, *habitats*, *peat lands*, *Oltenia*, *Romania*.

## ABSTRACT

*Due to its geographical position, Geopark Plateau Mehedinți lies in an area characterized by the alternation of oak and beech forests (beech to the N and W, oak regions to the S and SE), including secondary meadows with an abundance of species.*

*The phytogeographical character of the area - Carpathians Central European Flora type, with numerous infiltrations of sub-Mediterranean thermophilous plants present only in the southern extremity of the Geopark and a few endemic Dacian ones - gives distinctive characteristics, original, of this segment of South East Carpathians.*

*Following the current research, we reached a total of about 1260 taxa.*

## INTRODUCTION

Due to its geographical position, the territory lies in an area characterized by the alternation of oak and beech forests (beech to the N and W, oak regions to the S and SE), including secondary meadows with an abundance of species. The intersection of the temperate-continental, sub-Mediterranean, and even oceanic climates yields particular results, which are characteristic of this area. Its geological structure, unique to the Mehedinți Plateau, has led to a number of geological and speological peculiarities. Limestones cover a very large area of the territory and provide optimal conditions for an abundance of plants species.

**History**. The first plants from the territory were published by Borbás (1876, 1878). He botanized right along the austro-hungarian border on the 12.04.1873, expecting to find other plants to the east of the already famous and well-researched Domogled. Simonkai and Degen (Simonkai 1887; Soó 1928), had the same curiosity and also botanized here (Hunka Camena - a choronym we recently learned from an elderly woman). The samples they collected are now deposited in the National Museum in Budapest (BP). The same curiosity drove one of the authors of this paper (G. Negrean) from 1964 up to 1986, on several occasions, beginning with the Domogled and going beyond, with the hope that over the ridge additional species would be discovered. And indeed, Borbás's dream has now been fulfilled by us, for interesting botanical discoveries were made, which finally lead

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us to call the limestone Camena Mountain the "Little Domogled". Subsequently, we conducted extensive research throughout the whole Geopark, with the purpose of making a botanical and mycological monography. Prior to our research, there had been no special concerns for the flora of this territory. The great Romanian botanist, Dimitrie Grecescu, was present in the Geopark around 1898, but was just passing en route to the Mount Godeanu area. He published some plants from the slopes leading to the famous Țesna Valley, inside the Park, which really is a botanical paradise. Between 1963 and 2004, only 2 or 3 papers were dedicated to the Geopark, especially on the vegetation of the Lilac Forest in Ponoarele (Maloș & Firescu 1971; Măgălie 1970; Popescu et al. 2006; Popova-Cucu 1971). Sporadic data scattered throughout the Romanian literature emerged from occasional trips in the region (Bleahu et al. 1976; Maloș 1976). Our list, on the other hand, is based mostly on data we gathered ourselves, already totalling about 1,200 taxons and their number is enriched with every new research expedition we undertake to the Geopark. We will soon be in the fortunate position to publish several taxons which are new for Romania, which were discovered during our research trips in this territory.

The modern-day importance of our theme lies in the fact that this territory preserves a large range of habitat types and consequently, a complex biotic component. The purpose of the paper was to have an overall overview on the character of the flora, in light of recent floristic research.

## MATERIAL AND METHODS

We started by outlininig the boundaries of the territory to be researched. Observations were made on many choronymes of the Geopark, in different seasons, from April to November, between 2007 and 2011. Plant lists were made, while those that couldn't be determined on site had to be collected and put up for laboratory determinations. Habitats of community importance were identified and delimited. Nomenclature species, after Flora Romania (Săvulescu T. (ed.) 1952-1976), Flora Europaea (Tutin & al. 1993; Tutin & al. 1964-1980). Herbarium acronyms follow Holmgren et al. 1990.

## RESULTS AND DISCUSSIONS

In the Geopark there exist some areas that have been well protected for a long period of time and where human impact is insignificant. They are true natural habitat models, such as is the case with the Carstic systems and Natural Reserves. On all these territories we identified the following habitat types: Illyrian forrests of *Fagus sylvatica* (*Aremonio-Fagion*); Dacian *Geranium macrorrhizum* beech forests; Medio-European limestone beech forests of the *Cephalantherion-Fagion*; Illyrian oak hornbeam forests (*Erythronio-Carpinion*); *Tilio-Acerion* forests (residing) on steep slopes, debris and ravines; alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*) with *Stellaria nemorum* and *Iris pseudacorus*; sub-Mediterranean pine forests with endemic black pines (*Pinus nigra* subsp. *banatica*) with *Genista radiata*, shrubs of Carpatho – Balkanic ash (*Fraxinus ornus*), subcontinental peri-Pannonic scrubs of *Corylus avellana*, subcontinental peri-Pannonic scrubs of lilac (*Syringa vulgaris*) with *Genista radiata*, subcontinental peri-Pannonic scrubs of lilac (*Syringa vulgaris*) and ash (*Fraxinus ornus*), Carpatho – Balkanic *Juniperus sabina* scrubs, Carpatho – Balkanic scrubland of *Spiraea chamaedryfolia*, forrest edge communities with tall hydrophilic grasses starting at the plains level up to the montane and alpine meadows, screes with *Geranium macrorhizum*, *Geranium lucidum* and *Sedum fabaria*, calcareous rocky slopes with chasmophytic vegetation (*Asplenium trichomanes* subsp. *quadrivalens* and *Poa nemoralis*), calcareous rocky slopes with chasmophytic vegetation with *Draba lasiocarpa*

and *Ceterach officinarum*, Dacian-Balkanic communities residing on calcareous rocky slopes with chasmophytic vegetation with *Silene saxifraga* subsp. *petraea*, *Asplenium ruta-muraria* and *A. trichomanes* subsp. *quadrivalens*, Medio-European pioneer species communities on mobile calcareous screes, montane and alpine levels with *Achnatherum calamagrostis*, Carpatho – Balkanic communities of tall weeds with *Telekia speciosa* and *Petasites hybridus*, Carpatho – Balkanic meadows with *Agrostis capillaris* and *Festuca rubra*, semi-natural dry grasslands and scrubland facies meadows on calcareous substrates (*Festuco-Brometalia*), montane meadows and Carpatho – Balkanic meso-oligotrophic active raised bogs with *Eriophorum vaginatum* and *Sphagnum recurvum*.

The most important areas in terms of flora and fitocoenology are the following:

1. The Topolnița Cave area, the richest area containing special thermophilic species, many of which have meridional character. In the area above the entrance to the Topolnița Cave, one can find *Euphorbia myrsinites*, and also Carpatho – Balkanic bushes of ash (*Fraxinus ornus*). Near the same location, on rugged terrain, we identified the following species: *Draba lasiocarpa*, *Sesleria rigida*, *Tragopogon balcanicus*, *Himantoglossum hircinum* subsp. *caprinum*, *Verbascum vandasii*, *Hieracium troicum*, *Linum hologynum*, *Limodorum abortivum*, *Mercurialis ovata*, *Lactuca viminea* subsp. *viminea*, etc. In this area there are Daco-Getic communities of: *Sedum acre*, *S. sexangulare* and *Petrorhagia saxifraga*. Here we also identified the species *Bupleurum prealtum*, *Anthriscus nemorosa*, *Salvia amplexicaulis*, *Cirsium grecescui*, *Cirsium creticum*, etc.

The steep slopes, above Gaura lui Ciocârdie, a scarcely explored area, harbour Carpatho – Balkanic bushes of lilac (*Syringa vulgaris*) and ash (*Fraxinus ornus*). There are some other quite interesting plants: *Anthemis cretica*, *Asplenium scolopendrium*, *Alyssum petraeum*, *Carduus candicans*, *Chamaecytisus banaticus*, *Cotinus coggygria*, *Euonymus verrucosus*, *Fritillaria orientalis*, *Rosa tomentosa*, etc., including the dwarf shrub *Spiraea cana*. Not far from here, in the meadows of Jupâneşti, we found *Lathyrus linifolius*, the only (known) recent collection in the last 150 years in Romania.

The forests in the area mainly composed of *Fagus sylvatica* and *Aremonia agrimonoides* are particularly interesting: *Carpinus orientalis* coexists with *C. betulus* and *Fagus sylvatica*. The presence of *Carpinus orientalis* is explained by the continuous supply of seeds from the sunny walls, where *Carpinus orientalis* germinates and its seedlings frequently mature, reaching notable heights. Growing in the grassy layer are *Vinca herbacea*, *Arum maculatum*, rare plants in this area of the Carpathian Mountains.

The Polia (Pit) of Cireşu conceals shrubs at the forest edge, mainly *Prunus spinosa* on the southern slopes. In their vicinity grows a very diverse grassland flora, both at the bottom of the pit and on the surrounding hills. Noteworthy here are the many species of *Trifolium*: *T. arvense*, *T. aureum*, *T. hybridum* subsp. *elegans*, *T. incarnatum* subsp. *molinerii*, *T. dubium*, *T. ochroleucon*, *T. pallidum*, *T. pannonicum*, *T. patens*, *T. pratense* s. l., *T. repens* subsp. *repens*, *T. resupinatum*, *T. striatum*, then orchidacee species: *Orchis palustris* subsp. *elegans*, *Dactylorhiza incarnata* subsp. *incarnata*, *Orchis coriophora* s. l.; many individuals of *Ornithogalum sphaerocarpum* (at the extreme eastern limit of its area) and *Rumex thyrsiflorus*, a plant whose area extends from Eastern Europe to Denmark and the Netherlands; in the Flora of Romania it has been cited from 3 locations, to which some were later added in Roumanian Flora from Moldova and Transylvania. Also, *Linum flavum* was found, a population with many individuals, residing on a slope with southern exposition, *Linum hologynum*, *Odontites lutea*, and in the Lunca Topolniței (the area surrounding the Topolnita River), *Taraxacum palustre*.

Rare and endangered species from the Topolnița area: *Cerastium banaticum* (Rochel) Heuffel subsp. *banaticum*, *Cirsium acaule* Scop. subsp. *acaule*, *C. creticum*

(Lam.) d'Urv., *C. grecescui* Rouy, *Dactylorhiza incarnata* (L.) Soó subsp. *incarnata*, *Delphinium fissum* Waldst. & Kit. subsp. *fissum*, *Dianthus banaticus* (Heuffel) Borbás, *Echinops bannaticus* Rochel ex Schrad., *Euphorbia myrsinites* L., *Gymnadenia conopsea* (L.) R. Br., *Himantoglossum hircinum* (L.) Sprengel subsp. *caprinum* (Bieb.) Sundermann, *Jovibarba heuffelii* (Schott) Á. & D. Löve, *Lactuca viminea* (L.) J. & C. Presl subsp. *viminea*, *Limodorum abortivum* (L.) Swartz, *Linum hologynum* Reichenb., *Lunaria annua* L. subsp. *pachyrhiza* (Borbás) Hayek, *Luzula forsteri* (Sm.) DC., *Mercurialis ovata* Sternb & Hoppe, *Micromeria pulegium* (Rochel) Bentham, *Moenchia mantica* (L.) Bartl. subsp. *mantica*, *Neottia nidus-avis* L., *Orchis coriophora* Lam. subsp. *coriophora*, *O. coriophora* Lam. subsp. *fragrans*, *O. laxiflora* Lam. subsp. *elegans* (Heuffel) Soó, *O. morio* L. subsp *picta* (Loisel.) K. Richt., *O. ustulata* L., *Ornithogalum sphaerocarpum* A. Kerner, *Piptatherum virescens* (Trin.) Boiss., *Rumex thyrsiflorus* Fingerh., *Salvia amplexicaulis* Lam., *Seseli rigidum* Waldst. & Kit. subsp. *rigidum*, *Taraxacum janchenii* Kirchner & Štěpánek, *Tragopogon balcanicus* Velen., *Verbascum vandasii* (Rohlena) Rohlena.

2. The Camena Rocks, in the vicinity of which Borbás Vincentio botanized on 12.04.1873, shelter Carpatho – Balkanic *Juniperus sabina* scrubs, subcontinental peri-Pannonic scrubs of lilac (*Syringa vulgaris*) with *Genista radiata* (limited area habitat, probably endemic, including extremely rare plant species), subcontinental peri-Pannonic scrubs of lilac (*Syringa vulgaris*) and ash (*Fraxinus ornus*), southeast Carpathian scrubland of *Spiraea chamaedryfolia*, Carpatho – Balkanic shrubs of ash (*Fraxinus ornus*), subcontinental peri-Pannonic scrubs of *Corylus avellana*, calcareous rocky slopes with chasmophytic vegetation (*Asplenium trichomanes* subsp. *quadrivalens* and *Poa nemoralis*), calcareous rocky slopes with chasmophytic vegetation with *Draba lasiocarpa* and *Ceterach officinarum*, calcareous rocky slopes with chasmophytic vegetation with *Silene saxifraga* subsp. *petraea*, *Asplenium ruta-muraria* and *A. trichomanes* subsp. *quadrivalens*. The Camena forest area falls into the following types of habitats: Illyrian *Fagus sylvatica* forests (*Aremonio-Fagion*), Dacian forests of beech (Fagus sylvatica) and hornbeam (*Carpinus betulus*) with *Cardamine bulbifera*, Balkanic beech (*Fagus sylvatica*) forests with *Geranium macrorrhizum* and alluvial forest with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*) with *Stellaria nemorum*.

There are also fragments of submediterranean pine forests with endemic black pines (*Pinus nigra* subsp. *banatica*) with *Genista radiata*, *Spiraea cana*, *Hypericum rochelii*, *Jurinea glycacantha*, *Alyssoides utriculata*, *Sorbus banatica*, *S. borbasii*, *S. graeca*, *Asperula purpurea*, *Athamanta turbith* subsp. *hungarica*, *Centaurea atropurpurea* subsp. *atropurpurea*, *Echinops bannaticus*, *Gentiana cruciata* subsp. *cruciata*, *Hepatica nobilis*, *Micromeria pulegium*, *Sedum cepaea*, *Thlaspi dacicum* subsp. *banaticum*, *Tragopogon balcanicus*, *Epipactis helleborine*, *E. purpurata*, *Orchis mascula* subsp. *signifera*, *Dactylorhiza sambucina* f. *purpurea* and f. *bracteata*.

On the Camena Valley we also found hydrophilous tall herb fringe communities of plain and of the montane to alpine levels of *Petasites hybridus* with *Telekia speciosa*, eastern Carpathian yellow oatgrass meadows with *Agrostis capillaris* and *Festuca rubra*.

Rare and endangered species from the Camena area: *Abies alba* Miller, *Acinos alpinus* (L.) Moench subsp. *majoranifolius* (Miller) P.W. Ball, *Alyssoides utriculata* (L.) Medicus, *Arabis hornungiana* Schur, *Asperula purpurea* (L.) Ehrend. subsp. *purpurea*, *Athamanta turbith* (L.) Brot. subsp. *hungarica*, *Campanula grossekii* Heuffel, *C. lingulata* Waldst. & Kit., *Carlina acanthifolia* All. subsp. *acanthifolia*, *Centaurea triniifolia* Heuffel, *Cephalanthera longifolia* (L.) Fritsch, *Cephalaria laevigata* (Waldst. & Kit.) Schrader, *Cerastium banaticum* (Rochel) Heuffel subsp. *banaticum*, *Cirsium creticum* (Lam.) d'Urv., *C. grecescui* Rouy, *Dactylorhiza sambucina* (L.) Soó, *Dianthus banaticus* (Heuffel)

Borbás, *Delphinium fissum* Waldst. & Kit. subsp. *fissum*, *Echinops bannaticus* Rochel ex Schrad., *Edraianthus graminifolius* (L.) A. DC. subsp. *graminifolius*, *Ferula heuffelii* Griseb., *Festuca xanthina* Roemer & Schultes, *Galanthus nivalis* L., *Genista radiata* (L.) Scop., *Gymnadenia conopsea* (L.) R. Br., *Hypericum rochelii* Griseb. & Schenk, *Jovibarba heuffelii* (Schott) Á. & D. Löve, *Juniperus sabina* L., *Jurinea glycacantha* (Sibth. & Sm.) DC., *Lactuca aurea* (Schultz Bip. ex Pančić) Stebbins, *Lactuca viminea* (L.) J. & C. Presl subsp. *viminea*, *Linum uninerve* (Rochel) Jáv., *Listera ovata* L., *Lunaria annua* L. subsp. *pachyrhiza* (Borbás) Hayek, *Luzula forsteri* (Sm.) DC., *Mercurialis ovata* Sternb & Hoppe, *Micromeria pulegium* (Rochel) Bentham, *Moenchia mantica* (L.) Bartl. subsp. *mantica*, *Neottia nidus-avis* L., *Orchis coriophora* Lam. subsp. *coriophora*, *O. coriophora* Lam. subsp. *fragrans*, *O. laxiflora* Lam. subsp. *elegans* (Heuffel) Soó, *O. mascula* (L.) L. subsp. *signifera* (Vest) Soó, *O. morio* L. subsp *picta* (Loisel.) K. Richt., *Peltaria alliacea* Jacq., *Pinus nigra* Arnold subsp. *banatica* (Borbás) Novák, *Piptatherum virescens* (Trin.) Boiss., *Poa pumila* Host, *Primula elatior* (L.) Hill subsp. *leucophylla* (Pax) H.–Harrison ex W.W. Sm. & Fletcher, *Seseli rigidum* Waldst. & Kit. subsp. *rigidum*, *Silene flavescens* Waldst. & Kit., *S. nutans* L. subsp. *dubia* (Herbich) Zapał., *S. saxifraga* L., *Sorbus banatica* Borbás, *S. graeca* (Spach) Kotschy, *Tanacetum macrophyllum* (Waldst. & Kit.) Schultz Bip., *Taraxacum janchenii* Kirchner & Štěpánek, *Thlaspi dacicum* Heuff. subsp. *banaticum* (R. Uechtr.) Jáv.

3. The Limestone rocks at Isverna. The most noticeable formation here is "Potcoava" (The Horseshoe), located above the Isverna cave that's unaccessible, vertical walls resemble to an amphitheater. The Horseshoe can be approached through a single point, a place of inestimable beauty and also one of great floristic richness, with representative species like: *Cephalaria laevigata*, *Asperula capitata*, *Asplenium lepidum*, *Ferula heuffelii* (specimens about 2 m high), *Juniperus sabina*, *Epipactis atrorubens*, *E. helleborine*, *E. microphylla*, *Dianthus petraeus*, *Edraianthus graminifolius*, *Gagea spathacea*, *Centaurea atropurpurea*, etc.

Remarcably beautiful here, is also the Cănişa trail - a passage climbing towards the Izvorul Valea Alba (White Valley Springs) where one can admire species such as: *Gymnocarpium dryopteris*, *Asplenium ruta-muraria*, *Erysimum odoratum*, *Epilobium collinum*, *Senecio squalidus*, sothward oriented narrow limestone passages with: *Tragopogon balcanicus*, *Cephalaria laevigata*; and finally south-eastern and northern slopes with *Euphrasia illyrica* and *Sedum album*. On the descent to Vintilani, one notices the striking presence of a large population of *Cirsium acaule*, having the highest population density in the area.

Rare and endangered species from the Isverna area: *Asplenium lepidum* C. Presl, *Athamanta turbith* (L.) Brot. subsp. *hungarica* (Borbás) Tutin, *Centaurea atropurpurea* Waldst. & Kit., *Cephalanthera rubra* (L.) Rich., *Cephalaria laevigata* (Waldst. & Kit.) Schrader, *Cerastium banatictum* (Rochel) Heuffel subsp. *banaticum*, *Carlina acanthifolia* All. subsp. *acanthifolia, Delphinium fissum* Waldst. & Kit. subsp. *fissum*, *Digitalis ferruginea* L., *Edraianthus graminifolius* (L.) A. DC. subsp. *graminifolius*, *Epipactis atrorubens* (Hoffm.) Besser, *E. helleborine* (L.) Crantz, *E. microphylla* (Ehrh.) Sw, *Ferula heuffelii* Griseb., *Festuca xanthina* Roemer & Schultes, *Gymnadenia conopsea* (L.) R. Br., *Himantoglossum hircinum* (L.) Sprengel subsp. *caprinum* (Bieb.) Sundermann, *Jovibarba heuffelii* (Schott) Á. & D. Löve, *Lactuca viminea* (L.) J. & C. Presl subsp. *viminea*, *Linum hologynum* Reichenb., *Lunaria annua* L. subsp. *pachyrhiza* (Borbás) Hayek, *Micromeria pulegium* (Rochel) Bentham, *Orchis laxiflora* Lam. subsp. *elegans* (Heuff.) Soó, *O. morio* L. s. l., *O. militaris* L., *O. tridentata* Scop., *O. ustulata* L., *Peltaria alliacea* Jacq.

5. At the Piatra Pinilor (Pine Rock), in a quite limited area, thermophilic species grow, many of them with Balkan spreading: *Primula auricula* subsp. *serratifolia*, *Sorbus cretica*, *Genista radiata*, *Micromeria pulegium*, *Linum uninerve*, *Orchis militaris*, *Neottia nidus-avis*, *Piptatherum virescens*, *Atamantha turbith* subsp. *hungarica*, *Erysimum odoratum*, *Asperula tinctoria* subsp. *hungarorum*, *Festuca xanthina*, *Draba lasiocarpa*, *Seseli rigidum* s. l., *Silene flavescens*, *Saxifraga adscendens* f. *ramosissima*, *Arabis procurrens*, *Cotoneaster tomentosus*, *Clematis recta*, *Cotinus coggygria*, *Carex digitata*, *Teucrium montanum*, *Draba lasiocarpa*, etc.

6. Ponoare Zaton and Podul Natural (Natural Bridge), lapiés, or the Lilac forest, with a noticeable biodiversity (*Orchis simia*, *Erythronium dens-canis*, *Euphorbia lingulata*, *Listera ovata*, *Lactuca viminea* subsp. *viminea*, *Petrorhagia saxifraga*, *Piptatherum virescens*, *Tragopogon balcanicus*, etc.)

7. We also need to mention the relatively small diversity, but having great specificity, of the serpentine, which occur quite frequently in the Geopark (Ciolanul Mare, Ciolanul Mic, Pietrele Vinete, Giurgiani, Işfani, Rudina, etc.), which is probably a unique situation in Romania. The species that are characteristic to this ultrabasic substrate are: *Armeria maritima* subsp. *halleri*, *Poa pumila*, *Plantago serpentina*, *Asplenium cuneifolium*, *Notholaena marantae*, *Achnatherum calamagrostis*, *Silene bupleuroides*, *Potentilla cinerea*, *Gentianella germanica*, *Genista januensis*, *Asplenium septentrionale*, *Asplenium trichomanes*, *Antennaria dioica*, etc.).

8. The Geopark Plateau Mehedinți is the southernmost point in the Southern Carpathians where swamps exists with a flora which is scarce because of the climate conditions but still retain some characteristic plants:

a) În the rocky vicinity of the Camena, under Mount Ciolanul Mare, there are several small lakes, with a specific flora (*Eriophorum angustifolium*, *Thelypteris palustris*, *Platanthera chlorantha*, *Dactylorhiza maculata* subsp. *maculata*, *D. m.* subsp. *transsilvanica*, etc.), but above all one eutrophic raised bog with *Menyanthes trifoliata*, a very rare plant in southern Romania, coexisting with *Sphagnum* species, *Molinia caerulea*, *Eleocharis carniolica*, *Carex vesicaria*, *Comarum palustre*, *Thelypteris palustris*, *Epipactis palustris*, *Parnassia palustris*, *Alisma plantago-aquatica*, *Scirpus sylvaticus*, *Carex nigra*, etc.

b) The Obârşia Cloşani oligotrophic peat at Poiana Mare and Poiana Mică (*Sphagnum* ssp., *Eleocharis carniolica*, *Peplis portula*, *Carex echinata*, *Vaccinium vitis-idaea*, *V. myrtillus*, *V. uliginosus* subsp. *uliginosus*, *Lycopodium clavatum*, *Molinia caerulea*, *Eriophorum vaginatum*, *Hypericum humifusum*, *Campanula serrata*, etc.)

In the territory:

- The Fir tree (*Abies alba*) is has a sporadic presence in the area.

- The *Taxus baccata* species is present in the area: Valea Morii - several individuals of *Pinus nigra* subsp. *banatica* and some of *Taxus baccata*, on the limestone bedrock and lapies, with *Rhamnus saxatilis* subsp. *tinctoria*, *Linum uninerve*, *Sesleria rigida*; at the Valea Domnişoarelor (Isverna) there is a surprisingly large population of yew (over 400 individuals). Here we also identified *Daphne mezereum* and *Doronicum columnae*.

- Much of the limestone of the Mehedinți Plateau and Mehedinți Mountains are either bare or covered with hawthorn, the European cornel, blackthorn, smoketree, juniper and lilac mixed with isolated individuals of beech. In many places, wild lilac forms true forests, famous as those on Cornete Isverna in thermophilic forests with *Fraxinus ornus*, *Syringa vulgaris*, *Crataegus* sp., etc., or on rocks, like the ones at Piatra Pinului, Nadanova and Ponoarele.

The status of Alien plants

It is interesting to note that in the Mehedinți Geopark the number of alien plants is small (about 40), especially if one considers the large area of the park. The main reasons could be the climatic conditions, the substrate and especially the lack of intense circulation, partly due to the lack of modernized roads. Also, the absence of railway lines, perhaps the most important means of penetration of foreign plants. We noticed the existence of a grandient, SE towards NW, according to which their number reducess significantly. Other causes could be the decline of agriculturally cultivated areas and also the higher altitude and therefore more severe climate.

We wish to comment on a few species. The two *Amaranthus* species (*retroflexus* and *powelii*) are quite common in crops. One of the most dangerous invasive plants, *Ambrosia artemisiifolia*, is present only alongside roads, in their trenches, and usually in very small numbers. The Balkanian species *Cirsium candelabrum* is more widespread while *Conyza canadensis* is one of the most common weeds in the Geopark.

Also, very frequent in the Geopark are the two subspecies of *Erigeron* (*annuus* and *strigosus*). Finally, one can find *Xanthium italicum*.

Some alien plants, usually very common in almost every other part of Romania, are quite scarce here. Such is the case of *Galinsoga ciliata* and *G. parviflora*, *Lycium barbarum*, *Oxalis europaea* and *O. stricta*, *Portulaca oleracea* subsp. *oleracea*, *Tanacetum parthenium*, and *Veronica persica*.

*Impatiens glanduligera*, very common in the meadows of Transylvania, recently appeared in the humid valleys of the Geopark but is still scarce for the moment. However, no one knows what the future trend will be.

*Juncus tenuis* is present, not yet in large numbers. We only found the species in 2-3 locations, embedded in natural meadows.

Some cultivated plants, such as *Cucurbita pepo*, *Helianthus annuus*, *Lycopersicon esculentum*, *Zea mays*, etc., have a fleeting presence, and disappearing quickly.

Some ornamental garden plants also appear frequently, originating in the gardens of the locals and spreading to the ditches of the nearby roads (*Hemerocallis*, *Coleus*, *Iris germanica*, *Sedum sarmentosum*).

## CONCLUSIONS

Following our research, we reached a number of about 1260 taxa. Thus, we could determine the phytogeographic character of the area with greater accuracy, concluding that it falls under the Carpathians Central European Flora type, with numerous infiltrations of sub-mediterranean thermophilous plants and a few endemic Dacian ones, such as *Cephalaria laevigata*, *Cirsium grecescui*, *Linum uninerve*, *Micromeria pulegium*, *Pinus nigra* subsp. *banatica*, *Primula auricula* subsp. *serratifolia*, *Sorbus borbasii*, *S. banatica*, giving an original and specific appearance to this segment of the South Eastern Carpathians.

The North-South area one notices the presence of southern thermophilic elements, which are present only in the southern extremity of the Geopark (*Hordeum bulbosum*, *Jasione heldreichii*, *Achillea coarctata*, *Crucianella angustifolia*, *Euphorbia myrsinites*, *Scabiosa argentea*, *Convolvulus cantabrica*, *Crupina vulgaris*, *Echium italicum*, *Medicago arabica*, *M. polymorpha*, *Minuartia hirsuta* subsp. *frutescens*). A dense population of *Ruscus aculeatus* was identified north of the city of Baia-de-Aramă. A very special character is given to the area by the massive presence of the serpentine.

Peat lands are particularly important not only in terms of their vegetation but also because they can provide insight into the evolution of postglacial vegetation in the area.

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# *RUMEX THYRSIFLORUS* IN ROMANIA

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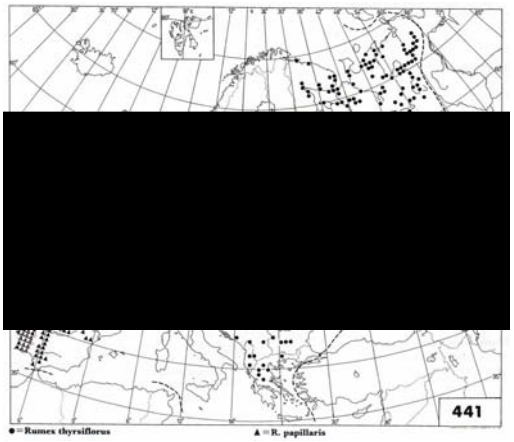
***Keywords***: *Rumex thyrsiflorus*, *rare plante*, *taxonomy*, *chorology*, *dioecia*, *Oltenia*, *Romanian Flora.*

## ABSTRACT

*Rumex thyrsiflorus is the new species for Mehedinți County and from Oltenia. The species is scientifically important, as rare and dioecious plant. In the paper make an antithetical description of the species Rumex thyrsiflorus and Rumex acetosa. Is indicated and completed chorology of the Rumex thyrsiflorus, shall be submitted some observations on the phenology of this species. From the observations, it follows that species Rumex tyrsiflorus is widespread, is found in many choronyms, with large populations, while the species Rumex acetosa was found only sporadically.*

## INTRODUCTION

*Rumex thyrsiflorus* Fingerh. (*Acetosa thyrsiflora* (Fingerh.) Á. Löve & D. Löve; *Rumex acetosa* L. subsp. *auriculatus* (Wallr.) A. Blytt & O. C. Dahl; *R. acetosa* var. *auriculatus* Wallr.; *R. acetosa* var. *crispus* (Roth) Čelakovský; *R. acetosa* var. *haplorhizus* (Czernjaev ex Turczaninow) Trautvetter; *R. acetosa* subsp. *thyrsiflorus* (Fingerh.) Hayek; *R. acetosa* Linnaeus subsp. *thyrsiflorus* (Fingerh.) Čelakovský; *R. auriculatus* (Wallr.) Murbeck; *R. haplorhizus* Czernjaev ex Turczaninow; *R. thyrsiflorus* var. *mandshuricus* A. Baranov & B. Skvortsov; *R. thyrsoides* auct.) – fam. *Polygonaceae* – is a plant of open habitats (meadows, weeds), of which area extends from the C, E Europe to Denmark and the Netherlands (Jalas & Suominen 1979) (Map 1).




Map 1. Distribution of *Rumex thyrsiflorus* species in Europa (from Atlas Florae Europaeae)

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În the Romanian Flora the plant is not cited from Mehedinți County. In this note we point to other choronyms for our country. Also we reviewed all the information we had at disposition from bibliography.

## MATERIAL AND METHODS

On the occasional trips in Mehedinți County, we identified the plant in many choronyms the Geopark Plateau Mehedinți. We determined the plant on living material; we made comparisons (all the living material) with the *Rumex acetosa* species.

Material has been collected from herbariums [BUC; CL; CRAI]. Herbarium abbreviation follow Holmgren et al. (1990). There have also been made photos of the plant in the original site (Ioana Ciortan).

The chorology map of the *Rumex thyrsiflorus* species in Romania was based on the literature data, consulting herbaria and field studies. The description of *Rumex thyrsiflorus* was antithetical to *Rumex acetosa*, species with which can be easily confused. It also presents the key to separating the two species.

## RESULTS

1a. Leaves blade oblong-lanceolate to lanceolate, 3-12 (-15) × 1-3 (-5) cm, usually more than 4 times as long as wide, with acute basal lobe, often bifidate. Inflorescences very compact, 1st-order branches usually repeatedly branched
*Rumex thyrsiflorus* Fingerh.

1b. Leaves blade oblong-ovate, ovate-lanceolate, to lanceolate, 4-10(-15) × 1-4(-6) cm, normally more than 2.5 times as long as wide, short basal lobe. Inflorescences usually lax, with 1st-order branches simple
*Rumex acetosa* L.

1. *Rumex thyrsiflorus* Fingerh. Perennial, dioecious plants, glabrous or nearly so. Rootstock vertical or oblique (reaching deep into substrate) and with rather 2d-order roots. Stem (Figure 1) usually erect, several from base, or occasionally solitary, grooved, branched in distal 1/2 (in inflorescence), (30-)40-100(-130) cm. Basal leaves with blade oblong-lanceolate to lanceolate, base sagittate or hastate (Figure 2), measuring 3-12 (-15) × 1-3 (-5) cm, usually more than 4 times as long as wide, both surfaces glabrous or veins minutely papillate;

Figure 1. *Rumex thyrsiflorus* - before flowering

Figure 2. *Rumex thyrsiflorus* – basal leaf detail

margins entire; apex acute; basal lobes (2) acute at apex, directed downward, ± parallel to petiole, or often reflexes outward, often bifidate. Cauline leaves small, to obscurely and irregularly repand, usually crisped and undulate, occasionally flat, apex acute, petiole short or nearly absent. Ochrea fugacious, white, membranous, often with fringed margins. Inflorescence terminal (Figure 3), occupying distal 3 of stem, usually dense, or interrupted in proximal part, broadly paniculate, pyramidal (1st-order branches usually repeatedly branched, with numerous 2d-order branches). Pedicels articulated near middle (articulation distinct), filiform, 2-7 mm. Flowers unisexual, (3-) 4-8 (-12) in whorls: male flowers - outer tepals erect, small; inner tepals elliptic, c. 2 mm.; female flowers - outer tepals reflexed in fruit; inner tepals enlarged in fruit; valves orbicular to broadly ovate, c. 2.5-3.5 (-4) × 2.5-3.5 mm, base rounded, truncate, or slightly cordate, apex obtuse; tubercles small or occasionally absent. Achenes black or dark brown, shiny, ellipsoid, trigonous, c. 1.5-1.8 × 0.8-1.2 mm, normally smooth. 2*n* = 14 (plants ♀), 15 (plants ♂) (PRODAN 1952; RECHINGER rev. by AKEROYD 1996; Flora of China; Flora of North America).

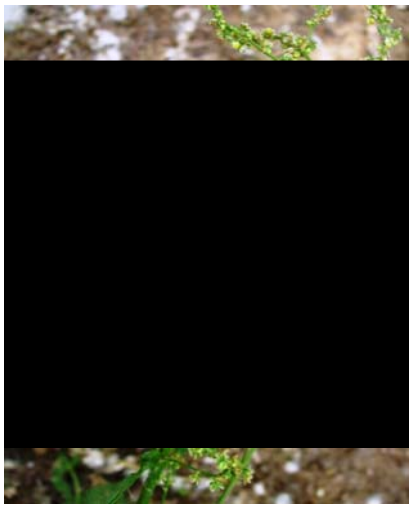

Figure 3. *Rumex thyrsiflorus* – inflorescence ♂

2. *Rumex acetosa* L. (*Acetosa pratensis* Miller; *Rumex acetosa* subsp. *pratensis* (Miller) A. Blytt & O. C. Dahl). Perennial, dioecious plants, glabrous or nearly so. Rootstock short and relatively thin, horizontal or slightly oblique rootstock (usually not reaching deep into substrate) and ± crowded 2d-order roots, or plant is rhizomatous. Stems erect, 40-100 cm, rarely ascending, usually simple or branched from the base, grooved. Basal leaves with blade oblong-ovate, ovate-lanceolate to lanceolate, c. 4-10(-15) × 1-4(-6), normally than 2.5 times longer as wide, short basal lobe at apices, base sagittate, margins entire, normally flat, apex acute or subacute. Cauline leaves small, petiole short or nearly absent. Ochrea fugacious, white, membranous, with fringed margins (especially in middle or upper leaves), 2.5 times longer than wide. Inflorescence terminal, paniculate, lax, and interrupted especially in proximal part, occupying distal 3 of stem branches reddish green, slender, simple or with a few secondary branches. Pedicels articulated near middle (articulation distinct), filiform, c. 2-5(-6) mm. Flowers unisexual, in whorls: male flowers: outer tepals erect, small; inner tepals elliptic, c. 3 mm; female flowers: outer tepals elliptic, reflexed in fruit; inner tepals enlarged in fruit; valves nearly orbicular (to broadly ovate), 3-4(-5) × 3-4 mm, base rounded or cordate, apex obtuse; tubercles small or occasionally absent. Achenes black to dark brown, 1.8-2.5 × 1.2-1.5 mm, shiny, smooth. 2*n* = 14 (plants ♀), 15 (plants ♂) (PRODAN 1952; RECHINGER rev. by AKEROYD 1996; Flora of China; Flora of North America).

In the Romanian Flora the species is cited in 3 places, have been added some from Moldova and Transilvania (OPREA 2005).

As a result of consulting literature as well as the original materials, we make the next chorology species in Romania: SM: *Lunca Someşului*, Vetiş, in herbosis (dig),

47°48′39,34″N, 22°45′24,43″E, alt. c. 115 m, 27 VII 2011, G. Negrean & C. Karácsonyi (N 16.591 - ♀) [BUC; BUCA; CL; HGN]. *Câmpia Ecedea*, Berveni N, in pratis, 47°45′50,08″N, 22°28′23,05″E, alt. c. 120 m, 25 VII 2011, G. Negrean & C. Karácsonyi (N 16.581 - ♀) [HGN]. *Câmpia Nirului*, Urziceni SW, Grădina Cailor, in fossa viam, 47°43′52,23″N, 22°22′30,92″E, alt. c. 117 m, 25 VII 2011, G. Negrean (N 16.566 - ♀) [BUC; BUCA; CL], idem ♂ (N 16.567) [BUC; CL]. Foieni W, Câmpul Iepurelui, in pratis humidis, 47°42′02.84″N, 22°20′02,83″E, alt. c. 129 m, 30 VII 2011, G. Negrean & C. Karácsonyi (N: 16.667 - ♀) [BUC; BUCA; CAREI; CL; HGN]. BH: Săcueni E, prope Lacul Vărgat, in herbosis, 47°21′07.258″N, 22°09′31.275″E, alt. c. 140 m, 27 VIII 2010, G. Negrean [CL; CAREI; CRAI]. Şimian NNW, in arenosis, 47°30′30.503″N, 22°04′50.932″E, alt. c. 130 m, 30 VII 2011, G. Negrean & C. Karácsonyi (N: 16.680 - ♀) [HGN]. CJ: Valea Someşului Mic near Apahida, in career of dacite (Prodan 1952: 388, 391). Apahida from Valea Someşului Mic (Oprea 1995: 46). MM: Borşa, between train station and water Vişeului (Prodan 1952: 388, 391), idem („I. Resmeriță & al., 1975-1987, Flora şi vegetația din Maramureş cu unele aspecte de ecologie şi bioproducție. mnsc., 370 pp." fide Oprea 2005: 46 & 643). BN: Năsăud (Bârlea 1991). BV: Şinca-Nouă (Ciocârlan 2009: 270). Piatra Mare Massif (Buiculescu 1989). MH: Gornenți NW, ad pedem Montes Ciolanul Mare, prope lacum, in pratis, 44°55′37,562″N, 22°30′39.661″E, alt. c. 870 m, 9 V 2011, Ioana Ciortan & G. Negrean [CL; CRAI]. Gornenți NW, in herbosis, 44°55′19.286″N, 22°32′00.214″E, alt. c. 800 m, 9 V 2011, Ioana Ciortan & G. Negrean [CL; CRAI]. Gornenți, centrum, 44°54′48.347″N, 22°32′45.343″E, alt. c. 610 m, 9 VI 2011, Ioana Ciortan & G. Negrean. Marga, in herbosis, 44°49′06.813″N, 22°35′25.886″E, alt. c. 600 m, 11 VI 2011, Ioana Ciortan & G. Negrean [CL; CRAI]. Cireşul SSW, in herbosis, 44°48′28.859″N, 22°31′54.371″E, alt. c. 410 m, 27 VIII 2010, G. Negrean [CL]. Cireşul ENE, Polia from Cireşu, in foenatis abruptis, 44°49′42.852″N, 22°33′26.651″E, alt. c. 390 m, 6 V 2011, Ioana Ciortan & G. Negrean [CL; CRAI]. Bunoaica centrum, in fossa viam, 44°47′43.385″N, 22°32′21.624″E, alt. c. 500 m, 9 VI 2011, Ioana Ciortan & G. Negrean. Bunoaica S, in schistosis, 44°47′19.531″N, 22°32′05.668″E, alt. c. 500 m, 14 VI 2011, Ioana Ciortan & G. Negrean. Schitul Topolniței, Schitul de Sus, 10 VI 2011, Ioana Ciortan & G. Negrean [CL; CRAI]. Bahna S, Valea Bahnei, in fossa viam, 44°46′07.328″N, 22°30′00.230″E, alt. c. 130 m, 11 VI 2011, Ioana Ciortan & G. Negrean [CL; CRAI]. GL: „Galați – Iacomi" (Ciocârlan 2000: 266; Ciocârlan 2009: 270). „GL: Iacomi gară" (Ciocârlan 2000, fide Oprea 2005: 46). Gohor (Mititelu et al. 1993). VS: basin of River Crasna (Vițalariu 1976; Mititelu & Huțanu 1996).

## DISCUSSIONS

*Rumex thyrsiflorus* is commonly misidentified as *Rumex acetosa*. The growth habit (stout, vertical rootstock), leaves with 2-fidate basal lobes, acute at apex, directed downward, ± parallel to petiole, or often reflexes outward, bifidate, middle cauline leaves curved inward (Figure 1), and pyramidal, usually much-branched panicle of *Rumex thyrsiflorus* (Figure 3) are traits especially useful for field identification. In addition, the inner tepals of *R. thyrsiflorus* are distinctly smaller than those of *Rumex acetosa*. From our observations that in the investigated *Rumex tyrsiflorus* species is widespread, being found in many choronyms with large populations, while the *Rumex acetosa* species was found only sporadic, scattered individuals.

At Cireşu, the plant grows in very rich hayfields, with: *Achillea crithmifolia, Aira elegantissima*, *Anthoxanthum odoratum*, *Asperula cynanchica*, *Brachypodium pinnatum*, *Briza media*, *Campanula persicifolia*, *Carduus candicans*, *Carex caryophyllea*, *Carlina*

*acanthifolia* subsp. *acanthifolia*, *Chrysopogon gryllus*, *Cirsium acaule* subsp. *acaule*, *Cleistogenes serotina*, *Cruciata pedemontana*, *Crupina vulgaris*, *Cynosurus cristatus*, *Danthonia alpina*, *D. decumbens*, *Dianthus giganteus* subsp. *giganteus*, *Ferulago silvatica*, *Galium album* subsp. *pycnotrichum¸ Inula hirta*, *Lathyrus hallersteinii*, *L. latifolius*, *Linum hologynum*, *Lychnis coronaria*, *Odontites lutea*, *Orchis coriophora* subsp. *fragrans*, *O. papilionacea*, *Petrorhagia saxifraga*, *Potentilla argentea*, *Rhinanthus rumelicus*, *Rubus canescens*, *Sanguisorba minor* subsp. *minor*, *Stellaria graminea*, *Tragopogon balcanicus*, *T. orientalis*, *Trifolium campestre*, *T. incarnatum* subsp. *molinerii*, *T. medium* s. l., *T. montanum*, *T. strictum*, *Vicia cassubica*, etc.

Importance. The plant is scientifically important, being rare and dioeciously plant. That we found it in large populations in Mehedinți County is coming to strengthen our conviction are the necessary further and detailed chorological research also research of the plant biology and ecology.

Limitative factors. Dioecia. From our observations it appears that male specimens are rarer. In the area investigated were identified many female exemplars. Male exemplars, few in fact, at their fructification, almost can not be identified, the flowers being dried.

Conservation measures. In the *Red Book of vascular plants in Romania* (Dihoru & Negrean 2009) it is recommended, among other things, carefully research and other areas to identify plants which could be confused with similar species - *Rumex acetosa*. Having regard to this, the plant should be grown in botanical gardens. Also is necessary to keep germs in gene banks. The species is preserved in the investigated area in some points were strictly protected.

The plant was introduced in the collection from Botanical Garden "Al. Buia" Craiova, Plant Systematic sectors, in the summer of 2011. She has adapted very well.

## CONCLUSIONS

The plant is more widespread in Romania, but do not was carefully observed. Attesting this many choronyms of Mehedinți County. Rigorous observations are needed on fenology and chorology species in other parts of the country.

## ACKNOWLEDGMENT

We thank the staff Geopark Plateau Mehedinți (Mr. Meilescu Cornel, Mrs. Monica Păunescu, Mr. Păunescu Sorin, Mr. Isverceanu Emilian and Mr. Chiliban Marius) for the support we have given the activity of land and beyond.

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**Vol. XVII ( LIII ) - 2012**

# INCIDENCE OF TUBERCULOUS MENINGITIS AT CHILDREN

Ciupeanu Călugăru Eleonora Daniela[1]

***Key words*:** tuberculuous menngitis, *Mycobacterium tuberculosis*, children

## ABSTRACT

*Research has been made during 2009-2011, at the Hospital "Dr. Victor Babes "Craiova. In this period of all cases of other forms tuberculosis 288, 17cases were TB meningitis.*

*During January 2009 - January 2011, at the Department of Pneumology Children's Hospital of Infectious Diseases "Dr. Victor Babes "Craiova were diagnosed 17 cases of meningitis tuberculosis.*

*The 17 cases of meningitis tuberculosis were made following laboratory tests supporting the diagnosis of meningitis tuberculosis: HLG, VSH, fibrinogen, IDR with 2U, PPD, chest X-ray PA bacteriological examination of sputum or gastric lavage for BK, bacteriological exam of LCR, computed tomography CT.*

## INTRODUCTION

Mycobacterium tuberculosis is the bacteria that causes tuberculosis (TB) has been present in the human population since antiquity - fragments of the spinal column from Egyptian mummies from 2400 BCE show definite signs of tuberculosis (Anastasatu C. et.al., 1971).

One of the most dreaded diseases of the 19th century, TB was the eighth leading cause of death in children 1 to 4 years of age during the 1920s. Tuberculous (TB) meningitis occurs when tuberculosis bacteria (*Myobacterium tuberculosis*) invade the membranes and fluid surrounding the brain and spinal cord. The infection usually begins elsewhere in the body, usually in the lungs, and then travels through the bloodstream to the meninges where small abscesses (called microtubercles) are formed. When these abscesses burst, TB meningitis is the result. In areas where TB prevalence is high, TB meningitis is most common in children aged 0 - 4 years, and in areas where TB prevalence is low, most cases of TB meningitis are in adults1t (Grzybowski S, et. al.,1975).

Diagnosis of TB meningitis is even more difficult than with other forms of bacterial meningitis. This is because it does not come on suddenly with classic meningitis symptoms. TB meningitis normally begins with vague, non-specific symptoms of aches and pains, low-grade fever, generally feeling unwell, tired, irritable, not being able to sleep or

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eat properly, and gradually worsening headache. This lasts for two to eight weeks. In the elderly, symptoms are even more subtle, often just drowsiness and feeling unwell (Corlan E., 1995).

It is not until weeks later that more obvious symptoms like vomiting, severe headache, dislike of lights, neck stiffness and seizures occur. Without medical treatment, the disease will progress causing confusion, obvious signs of nerve damage and eventually resulting in coma (Rieder HL.1999).

In the 20th century, the incidence of TB dropped dramatically in industrialised countries, and cases of TB meningitis decreased accordingly. TB and TB meningitis are major problems worldwide, and TB cases are on the rise in industrialised countries (Crofton J. et al.,1999).

In older infants and children, latent tuberculosis infection (LTBI), which is the first infection with the tuberculosis bacteria, usually produces no signs or symptoms.

## MATERIAL AND METHODS

As complications of tuberculosis are frequent in infancy, correct diagnosis of tuberculosis in infants is important.

Collecting of the pathological products has been made function the tuberculosis location from: sputum, pleural and peritoneal fluid, LCR. Sputum is collected by spontaneous emission, if the patient coughs or bronchial lavage. Pleural fluid, peritoneal fluid is collected through these cavities punctures, following the general rules of asepsis.

**Bacteriological diagnosis**

Direct exam shows an essential stage in the laboratory diagnosis of tuberculosis and involves performing direct smears of pathological product. Pathological products (especially sputum) are subject to prior homogenization by treatment with sodium hydroxide and stirring (product thin and uniform distribution of bacilli) and bacilli concentration in the upper layer with xylol and distilled water.

Fluorescence screening with auramine and rhodamine and ultraviolet examination (dots shining pale-green on the background of the field in case of the presence of Koch bacilli).

Positive samples should be controlled by Ziehl-Neelsen staining;

direct baciloscopy by Ziehl-Neelsen staining allows accurate diagnosis: red bacilli, arranged in cords, other elements (flora association, polymorphonuclear cells, epithelial integer) are blue.

**Isolation**

in vitro Lowenstein-Jensen medium with incubation at 370 C for 2 months;

in vivo: using male rats of 400 g, inoculated subcutaneously on the inner thigh of 1-2 ml pathological product.

**Identification**

Culture characters:

on Lowenstein medium: R colonies (ragged edges), dry, yellowish white, the human type increase after 14 days, the atypical rise in the first days and are colored.

Morphological characters:

culture smear (Ziehl-Neelsen color) highlights alcoolo-acid-resistant bacilli, red, sometimes disposed in cords with cord factor (pathogenicity factor) with epithelial cells, leukocytes, flora association, colored in blue.

Biochemical characters:

Catalase test differentiates atypical mycobacteria with a positive reaction from Koch bacillus, where the reaction is negative. The colonies obtained on solid media surface

are a covered with pirocathelar solution, hydrogen peroxide, and distilled water. After 2-5 minutes it's performed reading reaction. The reaction is positive if gas bubbles is observed.

## RESULTS AND DISCUSSIONS

Because infection with the TB bacillus can lead to delayed hypersensitivity to tuberculoproteins, testing for hypersensibility has been an important way to screen populations for tuberculosis infection and disease (Popa M., 2004).

The tuberculin test, called the Mantoux test, involves local injection of purified protein derivative (PPD), a standardized solution taken from culture fluids of Mycobacterium tuberculosis. The injection is done intradermally into the forearm to produce an immediate small bleb. After 48 and 72 hours, the site is observed for a red wheal called an induration, which is measured and interpreted as positive or negative according to size.

**Bacteriological exam**

Bacteriological examination is the main method for certainty diagnosis of TB.

Bacteriological diagnosis of tuberculosis in children is difficult and laborious because of the difficulty obtaining pathological products investigated (low expectoration) and paucibacilar character of biological products.

Microscopic examination and culture on solid or liquid medium remains routine methods for identification of mycobacteria.

**Microscopic examination**

Relatively accurate, rapid and inexpensive microscopic examination of Ziehl-Neelsen stained smear remains the usual method of identification of mycobacteria from biological and pathological products (spontaneous and induced sputum issued, bronchial aspirate and laryngo-tracheal secretions, gastric aspirate, CSF, urine, fluid pleural, pericardial, peritoneal, lung biopsy samples, bronchial lymph nodes).

Has epidemiological importance identifying sources intensely bacilifere and those contagious. Sensitivity of the method is low (22 -80%), positive results indicating products rich in bacilli (over 5000 -10 000 germs / ml). A microscopic examination positive for BAAR, whereas gastric aspirate in particular, it may not be possible that the alcohol resistant germs not be Mt.

**Examination by culture**

Considered the gold standard in the diagnosis of TB, culture can have a sensitivity of 80-85% and a specificity of 98%. Unfortunately confirmation by culture in children does not exceed 28 -30%.

Examination by culture has a much higher sensitivity than microscopy, it can detect the number of germs Mt whether the product under consideration is small-only 10 germs / ml, the sensitivity of the method depends on the number of samples examined, if one product is examined about 80%, the products examined by sensitivity may increase to 90%.

Mt cultivation requires 4-8 weeks to obtain the result (due to slow multiplication of Mt) positive culture is followed by the identification of Mt confirmation test case.

Culture media can be used as solid Lowenstein-Jensen, Ogawa Tebegent (with egg) Middlebrook 7 H10, 7 HI l (agar) or liquid as 7:12 Middlebrook, Kirchver, Youmons (with ox serum).

For each product analyzed are seeded 3 tubes of culture medium (9 tubes 3 products). Microscopic examination (M) and culture (C) is performed in specialized laboratories for bacteriological BK.

Research has been made during 2009-2011, at the Hospital "Dr. Victor Babes "Craiova. In this period of all cases of other forms tuberculosis 288, 17cases were TB meningitis.

During January 2009 - January 2011, at the Department of Pneumology Children's Hospital of Infectious Diseases "Dr. Victor Babes "Craiova were diagnosed 17 cases of meningitis tuberculosis.

The 17 cases of meningitis tuberculosis were made following laboratory tests supporting the diagnosis of meningitis tuberculosis: HLG, VSH, fibrinogen, IDR with 2U, PPD, chest X-ray PA bacteriological examination of sputum or gastric lavage for BK, bacteriological exam of LCR, computed tomography CT.

Cerebrospinal fluid (LCR) is obtained by lumbar puncture and represent the primary means of diagnosis of TB meningitis.

Cases of meningitis TB laboratory showed the following changes: presence of an inflammatory syndrome, tuberculin hiperegy, hilar and parahilar adenopathy, presence of Mt at the LCR bacteriological exam.

Data from the study of patients according to age shows a maximum number of patients consulted ranging from 11 to 15 years, and the lowest number of patients (3) was recorded for children under 5 years.

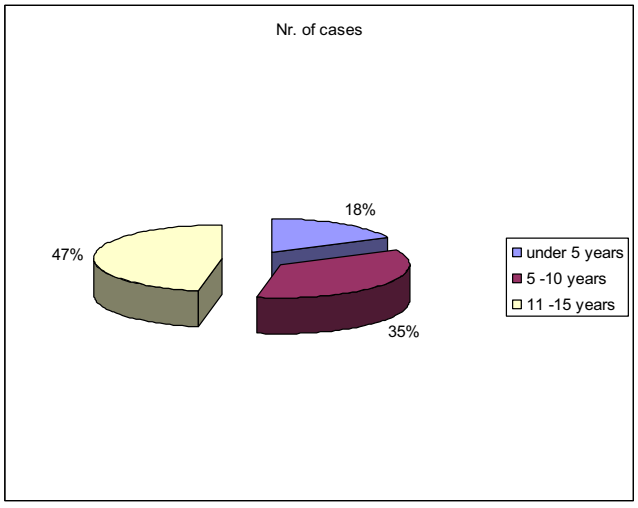


Figure 1. Distribution according to age

After analyzing patients according to sex we found a higher percentage of male 61.5%, comparative with female 41,18%.

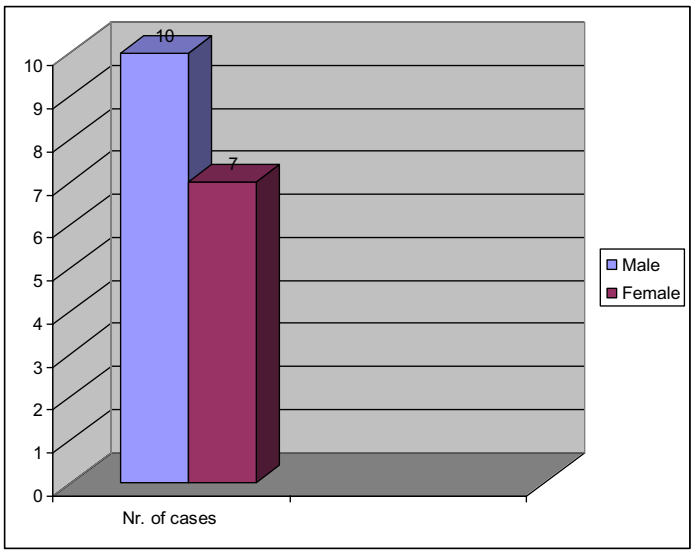


Figure 2. Distribution according to sex

As can be seen from the following graphic, out of 17 patients, 10 patients from the rural areas (77%) and 3 patients in urban areas (23%).

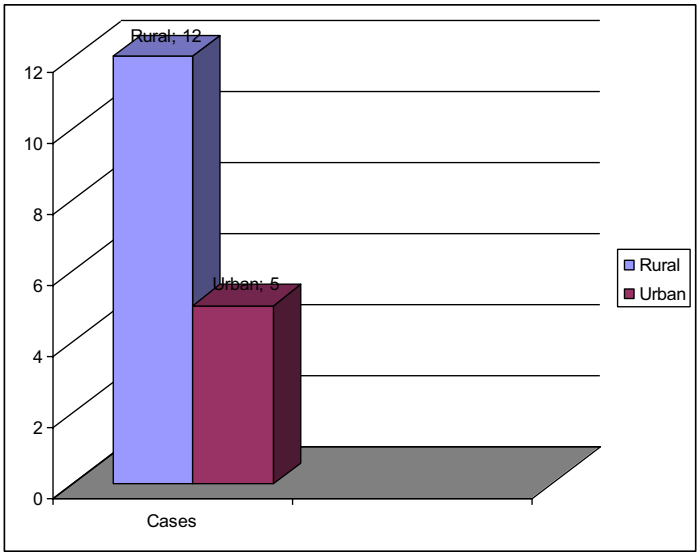


Figure 3. Sample distribution by area of origin

## CONCLUSIONS

From our research, regarding the incdence of meningitis tuberculosis in Oltenia area it come out Research has been made during 2009-2011, at the Hospital "Dr. Victor

Babes "Craiova. In this period of all cases of other forms tuberculosis 288, 17cases were TB meningitis.

From our data maximum number of patients (14) consulted ranging from 11 to 15 years, and the lowest number of patients (3) was recorded for children under 5 years.

After analyzing patients according to sex we found a higher percentage of male 61.5%, comparative with female 41,18%.

Regarding the distribution by area of origin, out of 17 patients, 10 patients from the rural areas (77%) and 3 patients in urban areas (23%).

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# STUDY OF BIOFILMS WITH HYDROXYAPATITE- POLY(METHYL METHACRYLATE)

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***Key words:*** *biofilms,* polymers , *hydroxyapatite-poly(methylmethacrylate)*

## ABSTRACT

*At the same time, with the increase and diversification of the use of polymeric materials, the attention of worldwide researchers has been focusing on the increasing of accumulation of solid waste that has an impact on the pollution of the environment. There have been many attempts using various techniques to develop high performance organic–inorganic hybrid materials, which combine the features of inorganic and organic substances. Organic polymer materials exhibit excellent flexibility, toughness, moldability, and adhesiveness, but their heat resistance properties are inferior to those of inorganic materials. To identify the structure and nanoparticles form in mixing HA - PMMA, we used the scanning electron microscopy (SEM) with the help of a QUANTA 133 from FEI Company. We performed the identification by comparing the images obtained for HA (hydroxyapatite) and PMMA (poly(methyl methacrylate)) with images for mixing HA-PMMA, we have a uniform structure, but however their low strength and brittle nature limits their potential applications to principally non-load-bearing applications*

## INTRODUCTION

The polymers with special properties represent the starting point for obtaining a wide range of new materials, which can be used in various fields of activity. At the same time, with the increase and diversification of the use of polymeric materials, the attention of worldwide researchers has been focusing on the increasing of accumulation of solid waste that has an impact on the pollution of the environment. The main alternative of control from the viewpoint of environmental pollution is the recycling process. An important problem raised by the recycling process is to maintain the polymer up to the performance level, which can allow its re-use in the same applications or in other important technological applications. There have been many attempts using various techniques to develop high performance organic–inorganic hybrid materials, which combine the features of inorganic and organic substances. Organic polymer materials exhibit excellent flexibility, toughness,

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moldability, and adhesiveness, but their heat resistance properties are inferior to those of inorganic materials. The materials properties of conventional organic–inorganic hybrid materials produced by mixing or dispersing inorganic materials in organic polymers are mainly determined by the nature of the interface between the organic and inorganic components, as well as by the size and dispersibility of the inorganic filler material.

*The purpose* of the present study was the preparation of hydroxyapatite-/poly (methyl methacrylate - obtained by recycling) (HA/PMMA) biofilm by interpenetrating polymerization of methyl methacrylate (MMA) monomer in the nanoporous HA templates.

## MATERIALS AND METHOD

HA was prepared by co-precipitation of calcium hydroxide ($Ca(OH)_2$) and phosphoric acid ($H_3PO_4$). (Synthesis to "Raluca Ripan" Chemistry Research Institute, Cluj-Napoca, Romania). The powders were annealed at 500 °C, for 2 hours. HA /poly(methyl methacrylate) nanocomposites were prepared by interpenetrating bulk polymerization of methyl methacrylate (MMA) monomer in structures of HA. The porous HA templates were prepared by mixing their calcined powders with poly(vinyl alcohol) (PVA) solution, shaping by uniaxial pressing and then firing at $1000^0$C. The templates were soaked in the solution mixture of MMA monomer and 0.1 mol% of benzoyl peroxide (BPO) for 24 h. The pre-composites were then bulk polymerized at $85^0$C for 24 h under nitrogen atmosphere. Crystalline phase and microstructure of the biofilm with hydroxyapatite-poly(methyl methacrylate) were characterized by scanning electron microscopy (SEM; FEI Company), respectively. To draw solid conclusions about the influence of the particles size ,form and uniform mixing on the chemical process I acquired PMMA sorted according to granulometric size.

## RESULTS AND DISCUSSIONS

Poly (methyl methacrylate) (PMMA), a transparent thermoplastic material, is an attractive polymer because of it's unique properties, in comparison with other polymers, such as: high mechanical strength, special optic properties, and durability. SEM micrographs of the obtained samples are shown in Figure 1. Scanning electron microscopy (SEM) was used to monitor the particle size and size distribution. One of the most highly compatible inorganic fillers is HA, the main inorganic component of bone,specifically nanometric HA. The mixing of nanofillers and polymers is difficult to achieve due to the large surface energy of nanometric particles. Hydroxyapatite (HA), was used in various biomedical fields such as bone substitute, dental material and hard tissue paste [8]. HA can accelerate the formation of bone-like apatite on the surface of implant [1].

Literature of speciality has been performed in recent years to determine the propensity of medical devices to sustain biofilm formation [5]. Several factors of the material, such as surface hydrophobicity [8], surface charge [6], surface roughness [7] and surface chemistry [2- 5,], have been shown to influence biofilm formation.

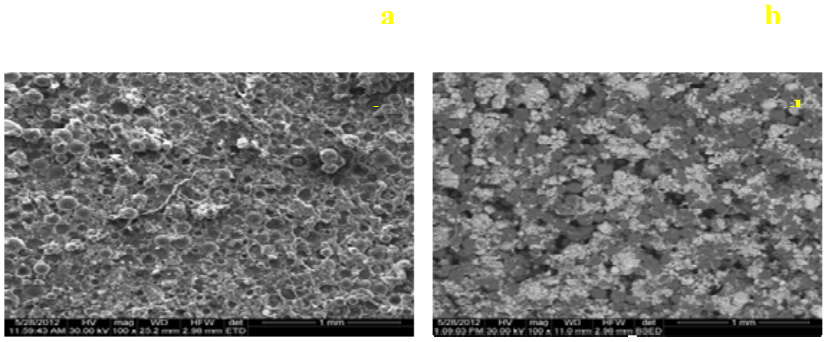


Figure 1. (a) SEM micrograph of as-received PMMA particles; (b) SEM micrograph of PMMA particles with nano hydroxyapatite - 20 wt.% HA, 80 wt.% PMMA; c) 30wt.% HA, 70wt%PMMA; d) 60wt.% HA, 40wt%PMMA; The bar 500 μm (top row) and 1mm (bottom row), SEM analysis

The particles of PMMA formed hard-spheres with a broad size distribution, from about 80 nm to 22 μm diameter, as shown by SEM micrographs (figure 1a – top row). It can be seen that some of the tightly packed spheres have connections between them. The PMMA (40)/HA(60) is shown in figure 1d with a 20μm and 5μm scale. Here there appears to be small spheres and the spheres look fractured because they were crushed with a pit and mortar for analysis. The largest particles are shown here to be about 25 μm diameter. Interestingly, there is clearly a different mechanism at work to generate an increased surface roughness on the spherical particles. Finally, figure 1a shows the PMMA (100), with a 500nm scale. The smallest particles formed are about 100nm in diameter. For all systems studied by SEM, there was a large distribution of particle sizes.

Generally, a uniform distribution of macro porous throughout the polymer matrices is found, but a solid film of polymer (skin layer) is observed over the majority of the sample surface. In this work, we have used PMMA beads instead of a polymer film. SEM pictures have shown that no external porosity was apparent in the beads (Fig. 1a), although no accessible internal porosity is likely present. On the contrary, PMMA polymer modified with MMA macro monomer have shown external porosity clearly evidenced by visual observation of SEM micrographs (Fig. 1b). Possibly, the presence of the macro monomer reduced polymer mobility and partially prevented the formation of the outer skin. Addition of filler particles to the polymeric systems may result in improved properties such as higher mechanical properties of the produced materials. Due to the importance of mechanical durability of porous mold materials under high pressure, it was attempted to

investigate the mechanical properties of the materials with the additional reinforcement particles. HA nanoparticles of various sizes have been incorporated by melt compounding in a poly(methyl methacrylate) (PMMA) matrix to enhance its mechanical properties. In order to improve nanoparticles dispersion, PMMA grafted particles have been prepared by atom transfer radical polymerization (ATRP) from well-defined HA nanoparticles. This strategy was expected to ensure compatibility between both components of the PMMA nanocomposites. SEM analysis have been performed to evaluate the nanohydroxiapatite dispersion.

## CONCLUSIONS

With the help of SEM a series of biofilm and nanocomposites have been characterisation. To investigate the role of HA in mixing with PMMA, causes the significant changes of structure nancompositelor. The addition of HA to PMMA (up to 40 wt%) did not change the bending properties significantly.

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# WHEAT MICOFLORA – WHEAT VARIETIES EXTENSION, MODELU LOCATION, CALARASI COUNTY

Cristea Carmen Mihaela[1], Berca M.[2]

***Keywords*:** *winter wheat, spring wheat,variety, pathogen, attack degree*

## ABSTRACT

*The researches identified the presence of pathogenic fungi Erysiphe graminis sp. f. tritici (powdery mildew), Mycosphaerella graminicola (septoria leaf spot), Leptosphaeria nodorum that produces septoria nodorum leaf and Giberella zeae that causes head blight. The attack of fungi Mycosphaerella graminicola and Leptosphaeria nodorum was remarked on winter wheat. Varieties Capo, Bitop, Fabula, and Philip presented powdery mildew attack on leaves. The highest attack of head blight was recorded by the variety Fabula. Variety Auradur recorded the lowest values of Mycosphaerella graminicola intensity attack (6.5%) and Leptosphaeria nodorum (4.5%).Variety Floradur showed powdery mildew and septoria leaf spot attack.The researches were carried out within doctoral thesis in POSDRU/107/1.5/S/76888.*

## INTRODUCTION

The foliar and ear diseases represent a risk agent for the wheat yield under Calarasi county conditions. The researces concerning foliar and ear pathogenic fungi are extremely wide, various and very important, so as the wheat crop.

The foliar and ear diseases complex include the following pathogens: *Erysiphe graminis* sp.f.*tritici*, responsible for appearance of the powdery mildew, *Mycosphaerella graminicola* (anamorph *Septoria tritici*), the pathogenic agent that causes septoria leaf spot, *Leptosphaeria nodorum* E. Muller (anamorph *Septoria nodorum*), that produces septoria nodorum blotch on leaves and glumes, *Gibberella zeae* (anamorph *Fusarium graminearum*) that produces head blight ( Cristea, 2005, Wiese , 1987).

The cultivation of resistent varieties to powdery mildew and septoria leaf spot represents an efficient way to prevent the attack of *Erysiphe graminis* sp.f.*tritici* (Cotuna et al., 2006, Moldovan et al., 2004.) and *Mycosphaerella graminicola* (Ahmed et al., 1994).

The instalation of head blight attack on winter wheat ears causes yield losses, both quantitative and qualitative and the selection of the varieties depending

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on their behaviour to the attack of pathogen *Fusarium graminearum* may reduce it's impact on production's efficiency (Ittu et al., 2010, Nishio et al., 2010). In the spring wheat varieties was detected their tolerance to the pathogen *Septoria tritici* (Zuckerman et al., 1997).

In this paper we present the observations regarding the reaction of some wheat varieties to the attack of pathogenic fungi *Erysiphe graminis* sp.f. *tritici, Mycosphaerella graminicola, Leptosphaeria nodorum* and *Gibberella zeae.*

## MATERIAL AND METHODS

The researches were conducted in year 2011-2012 at Modelu location, Calarasi, county, S.C. Probstdorfer Saatzucht , Romania SRL.

The aim of the researches is the identification of the pathogenic fungi and their attack on common winter wheat (*Triticum aestivum* L.) and on winter and spring durum wheat cultivars (*Triticum turgidum* ssp. *turgidum* conv. *durum*) under year 2011-2012 conditions.

The monitorized varieties were: Atrium, Arnold, Astardo, Balaton, Bitop, Capo, Fulvio, Fabula, Josef, Ludwig, Midas, Pireno, Philip, Pedro (common winter wheat varieties) and durum wheat varieties: Auradur (winter durum wheat) and Floradur (spring durum wheat).

There were found the pathogens: *Erysiphe graminis* sp.f. *tritici,*responsible of appearance of powdery mildew, *Mycosphaerella graminicola* (anamorph *Septoria tritici*) that produces septoria leaf spot, *Leptosphaeria nodorum* (anamorph *Septoria nodorum*) that causes septoria nodorum blotch on leaves and glumes and *Gibberella zeae* (anamorph *Fusarium graminearum*).

For the pathogenic foliar fungi it was noted the frequency and the attack's intensity and the attack degree was calculated using the calculation formula known in phytopatology domain. The seeds were treated with the product Amiral Profi, 0.5 liters/ton.

$F = \frac{nxN}{100}$ (%) where: n = no. of plants (organs) attacked; N = no. of plants (organs) observed;

$I = \frac{\sum (ixf)}{n}$ (%) where: I = % of plants (organ) atacked; f = no. of plants (organs) with given percent; n = noof plants (organ) atacked

$AD = \frac{FxI}{100}$ (%) where: F = frequency; I = the attack intensity; AD = attack degree;

In case of the pathogen *Gibberella zeae* it was noted the attack's frequency on ears.

## RESULTS AND DISCUSSIONS

After the observations effectuated in May 2011 it was identified the attack of the pathogenic fungi *Erysiphe graminis* sp.f. *tritici*, *Mycosphaerella graminicola* and *Leptosphaeria nodorum.*

The attack of *Erysiphe graminis* sp.f. *tritici* had the frequency of 100% but the intensity recorded values between 3,5% at variety Philipp and 10,5% at variety Capo. The value of the attack intensity has differentiated the monitorized varieties.

The attack of fungi *Mycosphaerella graminicola* and *Leptosphaeria nodorum* was frequent over foliar system at all the studied varieties.

The highest level of the attack was recorded by variety Josef, which had the AD=11,0%, in case of *Mycosphaerella graminicola* attack and AD=9,5% at variety Fulvio, in case of *Leptosphaeria nodorum attack* (table 1).

Table 1

Wheat foliar micoflora – winter wheat varieties extension, Modelu location, May, 2011-2012

| Variety | Pathogen | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Erysiphe graminis* sp.f.*tritici* | | | *Mycosphaerella graminicola* | | | *Leptosphaeria nodorum* | | |
| | F(%) | I(%) | AD(%) | F(%) | I(%) | AD(%) | F(%) | I(%) | AD(%) |
| Balaton | - | - | - | 100 | 9,5 | 9,5 | 75 | 6,5 | 4,9 |
| Atrium | - | - | - | 100 | 10,5 | 10,5 | 90 | 7,5 | 6,7 |
| Arnold | - | - | - | 100 | 9,5 | 9,5 | 70 | 8,0 | 5,6 |
| Fabula | 100 | 5,5 | 5,5 | 100 | 9,0 | 9,0 | 100 | 4,5 | 4,5 |
| Josef | - | - | - | 100 | 11,0 | 11,0 | 90 | 5,0 | 4,5 |
| Astardo | - | - | - | 100 | 9,5 | 9,5 | 90 | 4,0 | 3,6 |
| Bitop | 100 | 5,0 | 5,0 | 100 | 8,,0 | 8,0 | 90 | 4,5 | 4,0 |
| Midas | - | - | - | 100 | 8,5 | 8,5 | 80 | 6,0 | 4,8 |
| Peppino | - | - | - | 100 | 9,5 | 9,5 | 85 | 5,5 | 4,7 |
| Pireneo | - | - | - | 100 | 11,0 | 11,0 | 100 | 4,5 | 4,5 |
| Capo | 100 | 10,5 | 10,5 | 100 | 10,0 | 10,0 | 65 | 7,5 | 4,9 |
| Philipp | 100 | 3,5 | 3,5 | 100 | 8,5 | 8,5 | 70 | 8,0 | 5,6 |
| Fulvio | - | - | - | 100 | 8,0 | 8,0 | 100 | 9,5 | 9,5 |
| Pedro | - | - | - | 100 | 8,0 | 8,0 | 100 | 5,0 | 5,0 |
| Ludwig | - | - | - | 100 | 11,0 | 11,0 | 100 | 6,5 | 6,5 |

The data presented in table 2 shows that the variety Auradur was attacked by the pathogenes that produces septoria with a maximum frequency but with a much lower level of the intensity, of 3,5% in case of *Mycosphaerella graminicola* and 3,0% in case of fungus *Leptosphaeria nodorum*.

The variety Floradur prezented powdery mildew attack, of 4,0%, and attack of septoria leaf spot of 2,4%. It wasn't noticed the attack of *Leptosphaeria nodorum.*

Table 2

Wheat foliar micoflora – durum wheat varieties extension, Modelu location, May, 2011-2012

| Variety | Pathogen | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Erysiphe graminis* sp.f. *tritici* | | | *Mycosphaerella graminicola* | | | *Leptosphaeria nodorum* | | |
| | F(%) | I(%) | AD(%) | F(%) | I(%) | AD(%) | F(%) | I(%) | AD(%) |
| Auradur (winter wheat) | - | - | - | 100 | 3,5 | 3,5 | 100 | 3,0 | 3,0 |
| Floradur (spring wheat) | 100 | 4,0 | 4,0 | 60 | 4,0 | 2,4 | - | - | - |

Table 3

Wheat micoflora – winter wheat varieties extension, Modelu location, June, 2011-2012

| Variety | Pathogen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Erysiphe graminis* sp.f.*tritici* | | | *Mycosphaerella graminicola* | | | *Leptosphaeria nodorum* | | | *Gibberella zeae* |
| | F(%) | I(%) | AD(%) | F(%) | I(%) | AD(%) | F(%) | I(%) | AD(%) | F(%) |
| Atrium | - | - | - | 100 | 12,5 | 12,5 | 100 | 9,5 | 9,5 | 7,5 |
| Arnold | - | - | - | 100 | 12,5 | 12,5 | 100 | 10 | 10 | 7,5 |
| Astardo | - | - | - | 100 | 11,0 | 11,0 | 100 | 9,0 | 9,0 | 7,0 |
| Balaton | - | - | - | 100 | 11,5 | 11,5 | 100 | 8,5 | 8,5 | 7,0 |
| Bitop | 100 | 7,5 | 7,5 | 100 | 9,5 | 9,5 | 100 | 6,5 | 6,5 | 4,0 |
| Capo | 100 | 12 | 12 | 100 | 13,0 | 13,0 | 100 | 8,5 | 8,5 | 7,0 |
| Fabula | 100 | 9 | 9 | 100 | 12,0 | 12,0 | 100 | 6,0 | 6,0 | 8,0 |
| Fulvio | - | - | - | 100 | 11,5 | 11,5 | 100 | 10,5 | 10,5 | 7,5 |
| Josef | - | - | - | 100 | 12,0 | 12,0 | 100 | 8,5 | 8,5 | 6,0 |
| Ludwig | - | - | - | 100 | 12,0 | 12,0 | 100 | 8,5 | 8,5 | 5,0 |
| Midas | - | - | - | 100 | 9,0 | 9,0 | 100 | 6,5 | 6,5 | 4,0 |
| Pedro | - | - | - | 100 | 9,5 | 9,5 | 100 | 7,0 | 7,0 | 6,0 |
| Peppino | - | - | - | 100 | 10,0 | 10,0 | 100 | 8,5 | 8,5 | 5,0 |
| Pireneo | - | - | - | 100 | 12,0 | 12,0 | 100 | 9,0 | 9,0 | 6,5 |
| Philipp | 100 | 8,5 | 8,5 | 100 | 10,5 | 10,5 | 100 | 9,5 | 9,5 | 5,0 |

The results of the observations effectuated in June 2012 are presented in table 3 and 4. The data from table 3 shows that the value of the attack degree has increased at the identified pathogens, adding the frequency of the head blight on ears. The highest attack incidence of *G. zeae* was scored at varieties Fabula (F=8,0%), followed by the varieties Atrium, and Fulvio. The varieties Bitop and Midas recorded the lowest levels of head blight frequency on ears.

Concerning the variety Auradur, the frequency attack of head blight was of 4% and at variety Floradur it wasn't noticed the attack of fungus *G. zeae* (table 4).

Table 4

Wheat micoflora – durum wheat varieties extension, Modelu location, June, 2011-2012

| Variety | Pathogen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Erysiphe graminis* sp.f.*tritici* | | | *Mycosphaerella graminicola* | | | *Leptosphaeria nodorum* | | | *Gibberella zeae* |
| | F (%) | I (%) | AD (%) | F (%) | I (%) | AD (%) | F (%) | I (%) | AD (%) | F(%) |
| Auradur (winter wheat) | - | - | - | 100 | 6,5 | 6,5 | 100 | 4,5 | 4,5 | 4,0 |
| Floradur (spring wheat) | 100 | 6,0 | 6,0 | 100 | 7,5 | 7,5 | - | - | - | - |

## CONCLUSIONS

The foliar and ear micoflora at the tested varieties was compound from phytopathogenic fungi: *Erysiphe graminis* sp.f. *tritici* (powdery mildew), *Mycosphaerella graminicola* (septoria leaf spot), *Leptosphaeria nodorum* (septoria nodorum leaf and glumes blotch) and *Gibberella zeae* (head blight).

The common winter wheat varieties presented a higher attack degree of the identified pathogens than the durum winter wheat variety.

The durum spring wheat recorded powdery mildew and septoria leaf spot attack, being free of head blight attack.

The highest powdery mildew attack was determined at variety Capo. High levels of septoria were recorded by the variety Josef. The variety Fabula recorded the highest attack incidence of head blight.

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# THE EFFICACY OF THE TREATMENT ON SOME PATHOGENIC FUNGI IN WHEAT, MODELU LOCATION, CALARASI COUNTY

Cristea Carmen Mihaela[1], Berca Mihai[2]

***Keywords***: *winter wheat, variety, pathogen, attac degree, efficacy*

## ABSTRACT

*The aim of the researches was the determination of the treatments' efficacy in year 2011-2012 conditions on the attack of some pathogenic fungi in wheat, Erysiphe graminis sp. f. tritici (powdery mildew), Mycosphaerella graminicola (septoria leaf spot), Leptosphaeria nodorum (septoria nodorum leaf and glumes) and Gibberella zeae (head blight), at Modelu location, Calarasi county. The efficacy of the treatments was over 50% against powdery mildew and varied in case of septoria leaf spot attack between 45,5% at variety Astardo and 69,6% at variety Peppino. In case of Leptosphaera nodorum, the highest efficacy was of 68,4% at variety Philipp. The attack of head blight was reduced by 60% at varieties Bitop, Peppino, Ludwig. The researches were carried out within doctoral thesis in project POSDRU/107/1.5/S/76888.*

## INTRODUCTION

For Calarasi county conditions, the foliar and ear mycosis complex in wheat is a risk agent for yield and the crop rotation is an importatnt element for providing the health of plants and yield.

In the year 2011-2012 conditions, at Modelu location, on studied varieties, the foliar and ear mycosis complex included the pathogens: *Erysiphe graminis* sp.f.*tritici*, responsible of powdery mildew appearance, *Mycosphaerella graminicola* (anamorph *Septoria tritici*), the pathogen that causes septoria leaf spot, *Leptosphaeria nodorum* E. Muller (anamorph *Septoria nodorum*), that induces septoria nodorum leaf and glumes, *Gibberella zeae* (anamorph *Fusarium graminearum*) the fungus that produces head blight (Barbulescu et al ., 2002, Cristea, 2005, Wiese, 1987).

The chemical protection, combined with farm practices and the resistance of the varieties, are concerns for providing wheat yield (Nagy et al., 2004).

The researches on *Fusarium graminearum* show that it is involved in the appearance ofblack-point in wheat (Gheorghies et al., 2004, Cristea et al., 2008) and important studies highlight the quantitative and qualitative yield losses, as a result of the pathogen's attack (Stein et al., 2009).

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The applying of Tebuconazole treatment determined researches regarding the resistance of pathogen *Fusarium graminearum* on Tebuconazole, over mycotoxins production of the fungus (Rayco et al., 2010).

In this paper we present the observations regarding the efficacy of the treatments applied on winter wheat cultivated in year 2011-2012, at Modelu location, Calarasi county, on the attack of pathogenic fungi *Erysiphe graminis* sp.f. *tritici*, *Mycosphaerella graminicola, Leptosphaeria nodorum* and *Gibberella zeae.*

## MATERIAL AND METHODS

The researces were effectuated in year 2011-2012 at Modelu location, Calarasi county, S.C. Probstdorfer Saatzucht, Romania S.R.L.

The aim of the researches consisted in establishing the influence of the treatments applied in winter wheat varieties, on the attack of pathogenic fungi: *Erysiphe graminis* sp.f. *tritici,* the fungus responsible of powdery mildew appearance, *Mycosphaerella graminicola* (anamorph *Septoria tritici*) that produces septoria leaf spot, *Leptosphaeria nodorum* (anamorph *Septoria nodorum*) the agent that causes septoria nodorum leaf and glumes, and *Gibberella zeae* (anamorph *Fusarium graminearum*) that induces head blight.

The monitored varieties were: Atrium, Arnold, Astardo, Balaton, Bitop Capo, Fulvio, Fabula, Josef, Ludwig, Midas, Pireno, Philip, Pedro. There were appliedtwo treatments with the product Mystic Pro (Tebuconazole 200 grs/l + Prochloraz 300 grs/l ), in a dose of 1l/ha. The seed was treated with product Amiral Proffy 6 FS 0,5l/ ton. The experience was disposed in subdivided plots. For the leaf pathogenic fungi were noted the frequency and the attack intensity and was calculated the attack degree for the untreated and treated varieties, using the calculation formula known in the researches from phytopathology domain. On the basis of the observations, was calculated the efficacy of the applied treatments. In case of powdery mildew attack, it was noted the attack incidence on ear.

$F = \frac{n \times N}{100}$ (%) where: n = number of plants (organs) with attack; N = number of plants (organs) observed;

$I = \frac{\sum (i \times f)}{n}$ (%) where: I = % of plants (organ) attacked; f = number of plants (organs) with given percent; n = number of plants (organ) attacked;

$AD = \frac{F \times I}{100}$ (%) where: F = frequency; I = the attack intensity; AD= attack degree;

E = (Gant-Gat/Gant) x100% where:Gant= attack degree untreated variant;Gat= attack degree treated variant;

## RESULTS AND DISCUTIONS

The obervations effectuated after the second treatment show that the applying of tested product on monitored varieties has reduced significantly the attack of fungi. The product had an influence on the attack intensity of the monitored pathogens, and for the varietie Atrium, it has reduced the attack frequency in case of pathogen *Leptosphaeria nodorum* to 80% (Atrium). The incidence attack of fungus *Gibberella zeae* was reduced by half in the majority of winter wheat studied varieties. The attack of the pathogen was severe reduced at Bitop variety (table 1).

Table 1

The influence of the treatment on the attack of some pathogenic fungi in wheat, Modelu location, 2011-2012

| Variety | Product dose ( l/ha) | Pathogen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Erysiphe graminis* sp. f.*tritici* | | | *Mycosphaerella graminicola* | | | *Leptosphaeria nodorum* | | | *Gibberella zeae* |
| | | F (%) | I (%) | AD (%) | F (%) | I (%) | AD (%) | F (%) | I (%) | AD (%) | F (%) |
| Atrium | Mystic Pro 1l/ha | - | - | - | 80 | 6,0 | 4,8 | 70 | 5,5 | 3,8 | 4,0 |
| | Untreated | - | - | - | 100 | 12,5 | 12,5 | 100 | 9,5 | 9,5 | 7,5 |
| Arnold | Mystic Pro 1l/ha | - | - | - | 100 | 5,5 | 5,5 | 100 | 4,5 | 4,5 | 3,5 |
| | Untreated | - | - | - | 100 | 12,5 | 12,5 | 100 | 10 | 10 | 7,5 |
| Astardo | Mystic Pro 1l/ha | - | - | - | 100 | 6,0 | 6,0 | 85 | 4,5 | 3,8 | 3,5 |
| | Untreated | - | - | - | 100 | 11,0 | 11,0 | 100 | 9,0 | 9,0 | 7,0 |
| Balaton | Mystic Pro 1l/ha | - | - | - | 100 | 6,0 | 6,0 | 100 | 4,0 | 4,0 | 4,0 |
| | Untreated | - | - | - | 100 | 11,5 | 11,5 | 100 | 8,5 | 8,5 | 7,0 |
| Bitop | Mistic Pro 1l/ha | 80 | 3,0 | 2,4 | 100 | 4,0 | 4,0 | 80 | 3,5 | 2,8 | 1,5 |
| | Untreated | 100 | 7,5 | 7,5 | 100 | 9,5 | 9,5 | 100 | 6,5 | 6,5 | 4,0 |
| Capo | Mystic Pro 1l/ha | 100 | 5,5 | 5,5 | 100 | 7,0 | 7,0 | 100 | 4,5 | 4,5 | 4,5 |
| | Untreated | 100 | 12 | 12 | 100 | 13,0 | 13,0 | 100 | 8,5 | 8,5 | 7,0 |
| Fabula | Mystic Pro 1l/ha | 100 | 3,5 | 3,5 | 100 | 5,5 | 5,5 | 100 | 3,5 | 3,5 | 4,5 |
| | Untreated | 100 | 9 | 9 | 100 | 12,0 | 12,0 | 100 | 6,0 | 6,0 | 8,0 |
| Fulvio | Mystic Pro 1l/ha | - | - | - | 100 | 4,5 | 4,5 | 100 | 4,0 | 4,0 | 3,5 |
| | Untreated | - | - | - | 100 | 11,5 | 11,5 | 100 | 10,5 | 10,5 | 7,5 |
| Josef | Mystic | - | - | - | 100 | 5,0 | 5,0 | 100 | 4,0 | 4,0 | 2,5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pro 1l/ha | | | | | | | | | | |
| | Untreated | - | - | - | 100 | 12,0 | 12,0 | 100 | 8,5 | 8,5 | 6,0 |
| Ludwig | Mistic Pro 1l/ha | - | - | - | 100 | 5,0 | 5,0 | 80 | 6,0 | 4,8 | 2,0 |
| | Untreated | - | - | - | 100 | 12,0 | 12,0 | 100 | 8,5 | 8,5 | 5,0 |
| Midas | Mistic Pro 1l/ha | - | - | - | 100 | 3,5 | 3,5 | 100 | 2,5 | 2,5 | 2,0 |
| | Untreated | - | - | - | 100 | 9,0 | 9,0 | 100 | 6,5 | 6,5 | 4,0 |
| Pedro | Mystic Pro 1l/ha | - | - | - | 100 | 4,0 | 4,0 | 100 | 4,0 | 4,0 | 3,0 |
| | Untreated | - | - | - | 100 | 9,5 | 9,5 | 100 | 7,0 | 7,0 | 6,0 |
| Peppino | Mystic Pro 1l/ha | - | - | - | 100 | 3,5 | 3,5 | 100 | 3,0 | 3,0 | 2,0 |
| | Untreated | - | - | - | 100 | 10,0 | 10,0 | 100 | 8,5 | 8,5 | 5,0 |
| Pireneo | Mystic Pro 1l/ha | - | - | - | 100 | 5,0 | 5,0 | 100 | 3,5 | 3,5 | 3,0 |
| | Untreated | - | - | - | 100 | 12,0 | 12,0 | 100 | 9,0 | 9,0 | 6,5 |
| Philipp | Mystic Pro 1l/ha | 80 | 5,0 | 4,0 | 100 | 4,5 | 4,5 | 100 | 3,0 | 3,0 | 3,0 |
| | Untreated | 100 | 8,5 | 8,5 | 100 | 10,5 | 10,5 | 100 | 9,5 | 9,5 | 5,0 |

Table 2 contains the data regarding the calculation of applying treatments efficiency. It is observed the positive correlation between the influence of treatment on attack degree and treatment efficiency.

At varieties that recorded powdery mildew attack, the efficacy was over 54%, with the highest value of efficiency at variety Bitop (E=68%). In case of *Mycosphaerella graminicola* attack, the highest efficiency was calculated at varieties: Peppino (E= 69,6%), followed by Midas, Atrium and Fulvio with values of efficiency over 60%. The highest efficiency on the attack of *Leptosphaera nodorum* was calculated at variety Philipp, of 68,4%, followed by variety Peppino,with E= 64,7%.

The efficacy of treatment on fusarium attack was between 40% and 60%, with the highest value at varieties Bitop.

Table 2

The efficacy of treatment on the attack of some pathogenic fungi in wheat, Modelu location, 2011-2012

| Variety | Product dose ( l/ha) | Pathogen | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Erysiphe graminis* sp. f.*tritici* | | *Mycosphaerella graminicola* | | *Leptosphaeria nodorum* | | *Gibberella zeae* | |
| | | AD (%) | E (%) | AD (%) | E (%) | AD (%) | E (% | F (%) | E (%) |
| Atrium | MysticPro 1l/ha | - | - | 4,8 | 61,6 | 3,8 | 63,2 | 4,0 | 46,6 |
| | Untreated | - | - | 12,5 | - | 9,5 | - | 7,5 | - |
| Arnold | MysticPro 1l/ha | - | - | 5,5 | 56,0 | 4,5 | 55,0 | 3,5 | 50,0 |
| | Untreated | - | - | 12,5 | - | 10 | - | 7,0 | - |
| Astardo | MysticPro 1l/ha | - | - | 6,0 | 45,5 | 3,8 | 57,7 | 3,5 | 50,0 |
| | Untreated | - | - | 11,0 | - | 9,0 | - | 7,0 | - |
| Balaton | MysticPro 1l/ha | - | - | 6,0 | 47,8 | 4,0 | 52,9 | 3,0 | 57,1 |
| | Untreated | - | - | 11,5 | - | 8,5 | - | 7,0 | - |
| Bitop | MysticPro 1l/ha | 2,4 | 68,0 | 4,0 | 57,8 | 2,8 | 57,0 | 1,5 | 62,5 |
| | Untreated | 7,5 | - | 9,5 | - | 6,5 | - | 4,0 | - |
| Capo | MysticPro 1l/ha | 5,5 | 54,2 | 7,0 | 46,1 | 4,5 | 47,1 | 4,5 | 35,7 |
| | Untreated | 12 | - | 13,0 | - | 8,5 | - | 7,0 | - |
| Fabula | MysticPro 1l/ha | 3,5 | 61,1 | 5,5 | 54,2 | 3,5 | 41,6 | 4,5 | 43,7 |
| | Untreated | 9 | - | 12,0 | - | 6,0 | - | 8,0 | - |
| Fulvio | MysticPro 1l/ha | - | - | 4,5 | 60,9 | 4,0 | 61,9 | 3,5 | 53,3 |
| | Untreated | - | - | 11,5 | - | 10,5 | - | 7,5 | - |
| Josef | MysticPro 1l/ha | - | - | 5,0 | 58,3 | 4,0 | 53,0 | 2,5 | 58,3 |
| | Untreated | - | - | 12,0 | - | 8,5 | - | 6,0 | - |
| Ludwig | MysticPro 1l/ha | - | - | 5,0 | 58,3 | 4,8 | 43,5 | 2,0 | 60,0 |
| | Untreated | | - | 12,0 | - | 8,5 | - | 5,0 | - |
| Midas | MysticPro 1l/ha | - | - | 3,5 | 61,1 | 2,5 | 61,5 | 2,0 | 50,0 |
| | Untreated | - | - | 9,0 | - | 6,5 | - | 4,0 | - |
| Pedro | MysticPro 1l/ha | - | - | 4,0 | 57,8 | 4,0 | 43,0 | 3,0 | 50,0 |
| | Untreated | - | - | 9,5 | - | 7,0 | - | 6,0 | - |
| Peppino | MysticPro 1l/ha | - | - | 3,5 | 69,6 | 3,0 | 64,7 | 2,0 | 60,0 |
| | Untreated | - | - | 11,5 | - | 8,5 | - | 5,0 | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MysticPro 1l/ha | - | - | 5,0 | 58,3 | 3,5 | 61,1 | 3,0 | 53,8 |
| Pireneo | Untreated | - | - | 12,0 | - | 9,0 | - | 6,5 | - |
| Philipp | MysticPro 1l/ha | 4,0 | 52,9 | 4,5 | 57,1 | 3,0 | 68,4 | 3,0 | 40,0 |
| | Untreated | 8,5 | - | 10,5 | - | 9,5 | - | 5,0 | - |

## CONCLUSIONS

In the year 2011-2012 conditions, in Modelu location, the applying of tested product has reduced significantly the attack of monitored pathogens.

The efficacy on powdery mildew attack varied between 52,9 % at variety Philipp and 68% at variety Bitop.

The treatment had a positive influence by reducing the attack intensity, and in case of pathogen *Leptosphaeria nodorum,*at varieties Atrium, Bitop and Ludwig, alsoon frequency. Variety Peppino was remarked, with efficacies bigger than 60%, on monitored pathogens.

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# ANTIBIOTIC RESISTANCE OF BACTERIAL STRAINS ISOLATED FROM RESPIRATORY TRACT INFECTIONS

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Pencu Mirela[2]

***Key words:*** *staphylococci, upper respiratory infection, antibiogram, antibiotic resistance*

## ABSTRACT

*The resistance of pathogenic bacteria to the drugs which are commonly used in therapy of infectious diseases play an important role in medical world. The most common causes of upper respiratory tract infections are staphylococcal and streptococcal strains. In this paper we present the results of investigation about 3284 nasopharyngeal secretion samples from adult persons (Olt County, during two years) and about 1258 bacterial strains isolated from these samples. The staphylococcal strains (814) were tested through antibiogram for their antibiotic resistance. For the most part of tested strains, the bacteria presented high resistance to Tetracycline, Penicillin, Oxacillin and Erythromycin.*

## INTRODUCTION

The respiratory tract infections are mainly acute diseases and occur all year long, but most of all in wet and cold seasons. The frequency, intensity and symptoms hang on functionality of host respiratory system, performances of homeostatic and immune defence systems and mechanisms. The most part of respiratory diseases (95%) are upper respiratory infections, with minor clinical pattern, without fever and will not require hospitalization and medical consultation (Prajapati et al. 2011). The rest of all (5%) are lower respiratory infections and have severe clinical pattern. To all appearances around 80% of antibiotic consumption in community is for the treatment of respiratory tract infections (Aguilar et al. 2010).

The streptococci (*Streptococcus pyogenes*, *Streptococcus pneumoniae*) and staphylococci (*Staphylococcus aureus*) are the primary bacteria involved in upper respiratory tract diseases, some of them are a normal inhabitants of the human organisms and others possess some virulence factors for defeat the immune barrier of host. The high resistance of these bacteria to many antibiotic effects in respiratory tract diseases treatment increase the difficulty of infection control.

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Whereas the antibiotic resistance is increasingly regarded as an ecological problem (Aguilar et al. 2010), antibiotic resistance of pathogenic bacteria is an important subject for both the medical world and the biologists. Physiologic concentrations of some antibiotics may select resistant strains and destroy the susceptible ones. A high interest for antibiotic resistance phenomenon occurs because of several possibilities to transfer the resistance factors between different bacterial categories and because of emergence of high resistant bacteria through mutations (Wright 2010).

The antibiotic resistance of bacteria may be the presumption of treatment failure for severe bacterial infections. Because of this, is very important to determine the sensitivity or resistance to antibiotics of bacterial strains frequently involved in ordinary infections.

The antibiotic consumption is in association with selection of antibiotic resistant bacteria (Aguilar et al. 2010). Not only the pathogenic bacteria, isolated from the biological samples (from patients) present resistance to antibiotics, but also the bacteria isolated from natural environment (soil, water) (DebMandal et al. 2011).

The judicious use of antibiotics, only after medical recommendation and test the susceptibility of bacteria by antibiogram is the only way to maintain the relative control of antibiotic resistance phenomenon. Besides, this is also the strategy of European Commission, focused on antibiotic administration only by correctly medical prescription and for just reasons (Gilbert 2011).

In this paper we studied the resistance to antibiotics of bacterial strains isolated from upper respiratory tract infections, in adult subjects, in Olt County, during two years (2010 and 2011). It was establish the incidence of upper respiratory tract infection in terms of investigated patient's gender, but also in terms of bacterial genus.

For each staphylococcal strain which was isolated from infections the antibiogram performed. Hereby was established the susceptibility or the resistance of isolated bacteria to frequently use antibiotic. For the streptococcal strains (*Streptococcus pyogenes*) was not necessary this method, because they still preserve the sensitivity to Penicillin and the most of therapy use this drug (Popa 2004).

## MATERIAL AND METHODS

The nasopharyngeal secretions samples were gathering from 3284 adult patients, both male and female, with residence in Olt County, in urban and rural areas. They came to Caracal Public Hospital laboratory and private medical laboratory "SC Elite Medical", 1702 patients in 2010 and 1582 patients in 2011 (Table 1).

Table 1.

Distribution of samples

| Year | 2010 | | | | 2011 | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| Trimester | I | II | III | IV | I | II | III | IV | |
| Hospital Lab | 186 | 205 | 197 | 235 | 231 | 178 | 183 | 170 | 1585 |
| Private Lab | 274 | 162 | 261 | 182 | 194 | 203 | 137 | 286 | 1699 |
| Total | 1702 | | | | 1582 | | | | 3284 |

The collected samples on sterile nasopharyngeal swab were inoculated on specific culture media for investigate the presence of pathogenic bacteria.

To relieve the haemolytic *Streptococcus* strain were used heart infusion agar with 5% sheep blood and we kept the inoculated plates at 37°C for 24 hours. The small

β haemolytic colonies were investigated to microscope (Gram stain) and tested at Bacitracin, based on the susceptibility of *Streptococcus pyogenes* to this drug (Buiuc & Negut 2009).

Because the presence of *Staphylococcus aureus* in upper respiratory tract is about 30% in general population (Buiuc & Negut 2009), analysis for these bacteria are performed if the physician request that. For this purpose we used different solid media, like blood agar or Chapman medium. The plates were kept for 24 hours at 37°C and the colonies were investigated to microscope (Gram stain) and were tested for coagulase.

Every strain of *Staphylococcus aureus* were tested by antibiogram for establish the susceptibility to antibiotics. The difusimetric method was performed. The Muller Hinton medium was used in sterile plates (4 mm depth). The inoculums with 0.5 optical density on Mc Farland scale was prepared in sterile saline solution. The reference strain *Staphylococcus aureus* ATCC 25923 was used, too. The following antibiotics were used: Cefuroxime (CXM), Ceftriaxone (CRO), Biseptol (Co-trimoxazole, SXT), Clarithromycin (CLA), Doxycyclin (DO), Amoxicillin (AMX), Erythromycin (E), Ampicillin/Sulbactam (SAM), Tetracycline (T), Penicillin (P), Oxacillin (OX), Chloramphenicol (C), Cephalexin (CEF), Clindamycin (CL). The inoculated plates were kept for 16 - 18 hours at 35 - 37°C after the antibiotic discs were placed onto the medium surface. The results indicate the level of bacterial susceptibility to antibiotic effects: sensitivity, intermediate sensitivity or resistance.

## RESULTS AND DISCUSSION

This study revealed that the incidence of upper respiratory tract infections was 44.65% in 2010 and 37.80% in 2011 in studied patients. The male subjects were more than female ones, both in 2010 and in 2011 (Table 2).

Table 2.

Incidence of respiratory tract infections in terms of gender of patients

| Gender of patients | 2010 – Positive samples | | 2011 – Positive samples | | Total |
|---|---|---|---|---|---|
| | *Streptococcus sp.* | *Staphylococcus aureus* | *Streptococcus sp.* | *Staphylococcus aureus* | |
| Female | 169 | 191 | 92 | 196 | 648 |
| Male | 174 | 226 | 109 | 201 | 710 |
| Total | 343 | 417 | 201 | 397 | 1358 |

The bacterial strains which were involved in positive cases belong to *Streptococcus* genus and to *Staphylococcus aureus* species. Both in 2010 an 2011, the staphylococcal infections were more than streptococcal infections (59.94% versus 40.05% in all studied period). These results confirm that the staphylococci and streptococci are commonly present in nasopharynx (Buiuc & Negut 2009) at asymptomatic subjects; for streptococci, the nasopharyngeal carriage range is from 10 to 40%, in age dependent manner (Aguilar et al. 2010).

The results of antibiograms for staphylococcal strains revealed a high sensitivity of bacteria to antibiotics from new generation (Cefuroxime, Cephalexin), since to old antibiotics (Tetracycline, Penicillin, Erythromycin) the resistance of bacteria was very explicit. The majority of bacteria involved in respiratory tract infection (upper or lower) are sensible to antibiotics of cephalosporin group (Slavcovici et al. 2006).

The sensitivity of bacterial strains is presented in Figure 1 and the resistance of bacterial strains to antibiotics is presented in Figure 3. Most of tested bacteria revealed also the intermediate sensitivity to Clarithromycin, Ampicillin/Sulbactam, Clindamycin, but not to Penicillin (Figure 2).

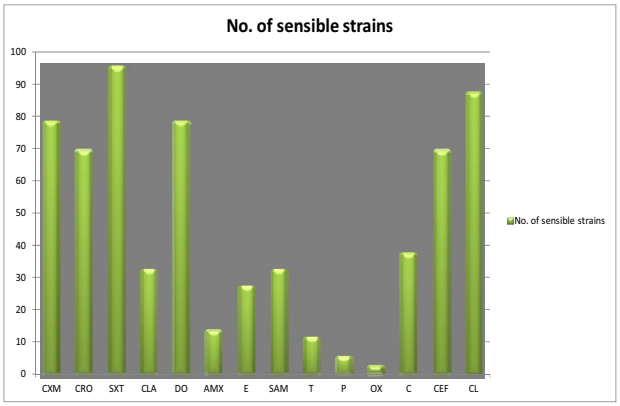


Figure 1. The sensitivity of bacterial strains to antibiotics

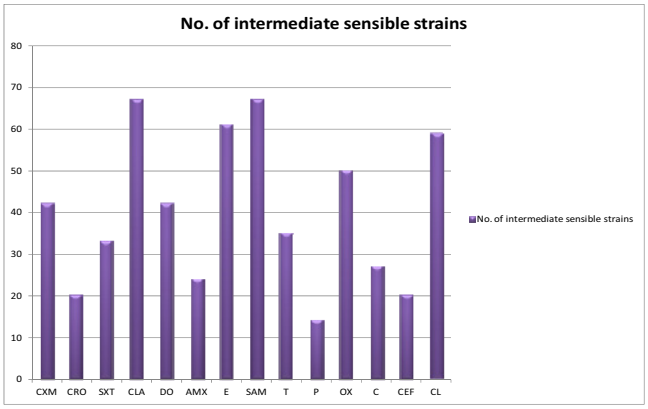


Figure 2. The intermediate sensitivity of bacterial strains to antibiotics

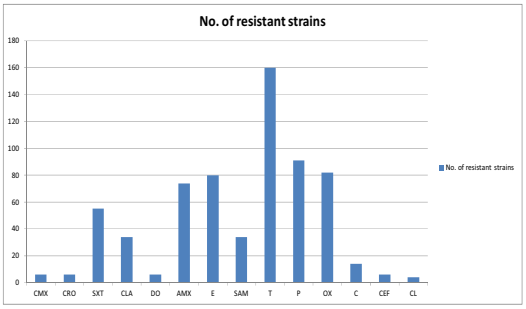


Figure 3. The resistance of bacterial strains to antibiotics

The resistance to Tetracycline was higher than other, 160 strains reveal that. Just 11 strains presented sensitivity to this drug, but more presented intermediate sensitivity. Another obvious antibiotic resistance was to Penicillin and Erythromycin.

Concerning the Methicillin (and Oxacillin) bacterial resistance, this study reveals many strains which possess it. The methicillin-resistant *Staphylococcus aureus* (MRSA) are involved in various infections, and usually they are multidrug resistant to antibiotics. This pathogenic profile due to complex treatment of infections with such strains, occasionally complicated with septicaemia and hospital-associated infections (Deurenberg & Stobberingh 2009).

## CONCLUSIONS

The staphylococcal and streptococcal strains are often implicated in upper respiratory tract infections, both in male and female subjects. The antibiotic resistance of staphylococcal strains was obvious to old ones (Tetracycline, Penicillin, Erythromycin) and to β - lactam antibiotics (like Oxacillin). An important sensitivity of studied strains was to antibiotics from new generations.

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# *HEPATICA TRANSSILVANICA* IN THE DAMBOVIŢA COMPLEX GORGES

Dinuţă Maria Violeta[1], Teodorescu A.[2]

***Keywords***: *Ranunculaceae, morphology, chorology, endemic*

**ABSTRACT**

*Hepatica transsilvanica Fuss is considered a sporadic species in Romania. It was identified in Dambovita gorges Complex. This paper will provide description, illustrations and topographic representation of the resorts has been identified in some gorges from the upper basin of river Dâmboviţa. The information presented uses personal data and literature.*

## INTRODUCTION

The genus *Hepatica* Mill. (Ranunculaceae family) consists of ten species with several varieties, perennials spread by shady areas, forests and shrubs from the cool temperate zones to the northern Hemisphere (Hara & Kurosawa 2005). In Europe, the genus is represented by three species, two of which are found only in Romania: *Hepatica nobilis* Schreb., *Hepatica transsilvanica* Fuss and *Hepatica x media* Simonk.

*Hepatica transsilvanica* Fuss is a vernal species, protheranthous plant, where flowers appear before leaves in early spring. The species *Hepatica transsilvanica* Fuss is a glacial relict that is distributed in deciduous forests in Eastern and Southern Carpathians.

Dâmboviţa Complex gorges, located in the upper basin of Dambovița are placed between Iezer-Păpuşa and Piatra Craiului Mountains.

Dambovita Complex gorges are the largest complex in the country with great landscape value that consists of 18 quays (Popescu-Argesel, 1980).

These quays have a length of 9 km, being located between Dealul Crucii Massif (West and North), Ghimbav Mount (East) and Fundul Neagului (South), up to Rucăr (Alexiu V., 1998a).

Varied relief generates vertical gradients in lower temperature, increasing the amount of precipitation, nebulosity and atmospheric pressure. The vegetal cover, especially forest vegetation, and slope inclination influence the variations in the local topoclimate.

The climate is characterized by moderate winters, with relatively warm summers and long, warm, dry autumns.

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The thermal regime is characterized by annual average of 4-8$^{o}$ C, with decreasing altitude temperature: 7.2°C at Rucar (700 m), 4.4$^{o}$C on the Fundata (1 370 m) and -2.8$^{o}$C at the Omu Peak (2505 m).

## MATERIAL AND METHODS

The investigation carry on *Hepatica transsilvanica* species, which are presented in this paper, were based on personal research and using the work of some authors (Alexiu V., 2003; Ciocarlan V., 2009; Monica Neblea, 2006). The fenotip of the species was identified by direct observation on morphological aspects, in its natural habitat. For taxonomical integration and synonymy setting it has been used Flora Europaea (Tutin, 1978) and *Flora ilustrată a României* (Ciocarlan V., 2009). To establish the species chorology it has been taken GPS coordinates. For orthophotomaps elaboration, the identified coordinates ware process in AutoCAD program. The species habitat was characterized by using *Habitatele din România* and *Manual de interpretare a habitatelor Natura 2000 din România* works (Donita et al., 2005;Gafta & Mountford, 2008).

## RESULTS AND DISCUSSION

Scientific name: *Hepatica transsilvanica* Fuss (Fig. 1)
Syn.: *Anemone transsilvanica* (Fuss) Heuff.; *Hepatica angulosa* auct., non (Lam.) DC
Popular name: Crucea voinicului
Scientific classification:
Kingdom: *Plantae*, Încrengătura: *Spermatophyta*, Division: *Magnoliophytina*, Class: *Magnoliopsida* , Order: *Ranales*, Family: *Ranunculaceae,* Genus: *Hepatica*
Description: the plant has 10-12 cm, a long horizontal or oblique rhizome, from which is forms every year, leaves and flowers. The leaves are double trilobite, with long, pubescent petiole. Flowers are blue, actinomorphic, with large petal (usually 10) and hairy peduncle. The Hepatica transsilvanica species blooms in April-May and its flowers (3-4 cm) are bigger than *Hepatica nobilis.* Fruits are pubescent achenes. Pollen has 5 pores (FLORA R.P.R, 1952-1976).

Fig. 1. *Hepatica transsilvanica* Fuss (orig.)

Origin and geographical distribution in Romania: *Hepatica transsilvanica* Fuss is mentioned by M. Fuss and F. Schur in Piatra Craiului Massif. Is a perennial plant that

grows only in Southern and Eastern Romanian Carpathian. It grows as an endemic species, in shady areas that are covert with forests and shrubs vegetation. Was found in beech forests near Brasov by botanist Karl George Kotschz. It was first replanted in the park of Schonbrunn (Vienna) and then to other places as St. Petersburg, Paris and London.

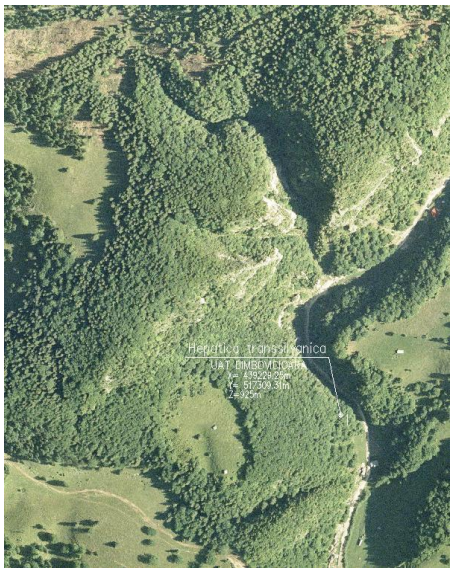


Figure 2. Orthophotomap *Hepatica transsilvanica* Fuss, Cheile Brusturetului (original)

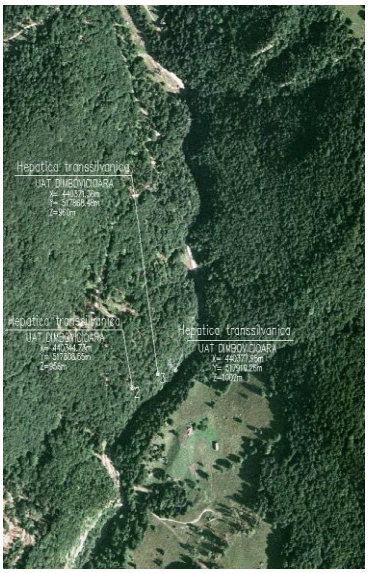


Figure 3. Orthophotomap *Hepatica transsilvanica* Fuss, Cheile Dambovicioarei (original)

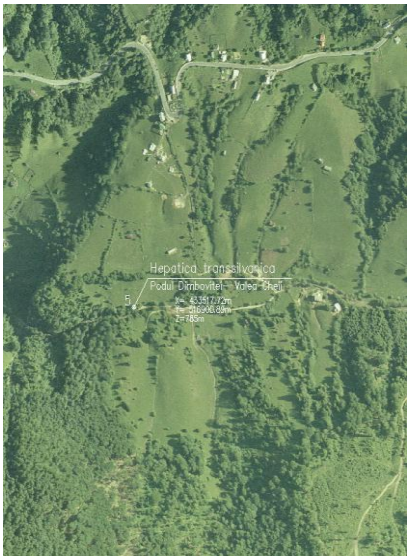


Figure 4. Orthophotomap *Hepatica transsilvanica* Fuss, Valea Cheii (original)

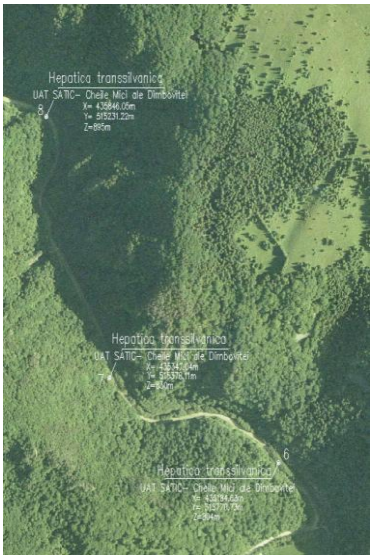


Figure 5. Orthophotomap *Hepatica transsilvanica* Fuss, Cheile Mici ale Dâmboviţei (original)

In România was identified in: Meridional Carpathians (Mountains Ţarcu, Leaota Mountains, Godeanu Mountains, Cernei Mts., Piatra Craiului Mts., Iezer-Păpuşa Mts.) and Oriental Carpathians (Hăşmaşu Mare, Hăşmaşu Mic Mts., Rarău Mts., Suhard Mts.).

The chorology in Arges county:

Râiosu-Buda Mountains: (Buia & Todor, 1948; Ileana Stancu, 2005);

Piatra Craiului Massif: Dâmbovicioara Valley (Greecescu, 1898; Mihailescu S., 2003), Dragoslavele (Savulescu, 1953);

Ghimbav Mountains: Valea Dâmboviţei (Danciu et al, 2006), Cheile Mari ale Dâmboviţei, Cheile Cheii, Cheile Ghimbavului, Cheile Rudăriţei (Alexiu V., 1998b; Monica Neblea 2007), Cheile Crovului (Pop O., 2006);

Iezer-Păpuşa Mountains: Cătun Mountains, Iezer Valley, Cheile Mici ale Dâmboviţei, (Alexiu V., 1998c);

Leaota Mountains: Cetăţeni (Alexiu V., 2004a);

Valea Vâlsanului: (Sanda V. et. al., 1995; Alexiu V., 2004b).

In superior basin of Dâmboviţa, *Hepatica transsilvanica* Fuss species was identified in the following quays: Cheile Mari ale Dâmboviţei, Cheile Brusturetului (Fig. 2), Cheile Dâmbovicioarei (Fig. 3), Cheile Ghimbavului, Cheile Cheii (Fig. 4), Cheile Mici ale Dâmboviţei (Fig. 5), Cheile Cheiţei, Cheile Rudăriţei and Cheile Prepeleacului.

Habitats Natura 2000:

8210 Calcareous rocky slopes with chasmophytic vegetation: Calcareous rocky slopes with chasmophytic vegetation, Dâmboviţa Complex Gorges, belongs to class *Asplenietea trichomanis,* order *Potentilletalia caulescentis,* alliance *Cystopteridion fragilis*, which is integrated in association *Asplenio trichomanis-Cystopteridetum fragillis*. This sciaphilc habitat is characterized by height diversity, with a large number of endemic species. In the floristic composition of the coenoses, find in Dâmboviţa Gorges, were identified a series of species: *Asplenium viride, Cystopteris fragilis, Gypsophila petraea, Saxifraga corymbosa, Saxifraga paniculata, Saxifraga cuneifolia, Valeriana montana, Cortusa matthioli, Asplenium ruta-muraria, Carex brachystachys, Moehringia muscosa, Sedum hispanicum, Asplenium trichomanes, Cardaminopsis arenosa, Poa nemoralis, Polypodium vulgare, Sedum maximum, Valeriana tripteris, Gymnocarpium robertianum.*

The importance of this habitat lies in the presence of some endemic species: *Aconitum moldavicum, Campanula carpatica, Hepatica transsilvanica, Thymus comosus.*

In Dâmboviţa Complex Gorges was mentioned the *Campanuletosum carpaticae* subassociation that contains some differential species: *Campanula carpatica, Hepatica transsilvanica, Saxifraga cuneifolia, Cortusa matthioli, Valeriana montana* (Donita et al., 2005; Gafta & Mountford, 2008).

## CONCLUSIONS

*Hepatica transsilvanica* Fuss is an endemic species that grows on calcareous substrate, being geographical isolated.

It is a vernal, proteranthous plant from Pleistocene that is protected by law. It has a sporadic distribution in Romania and vegetates only in Orientali and Meridionali Carpathians. The plant prefers the rocky, calcareous and moisture substrate.

*Hepatica transsilvanica* is included in Calcareous rocky slopes with chasmophytic vegetation, being integrated in *Asplenio trichomanis-Cystopteridetum fragillis association, campanuletosum carpaticae sub association.* In this association, beside *Hepatica*

*transsilvanica* were also identified some others endemic species: *Campanula carpatica, Saxifraga cuneifolia, Cortusa matthioli, Valeriana montana.*

## ACKNOWLEDGEMENTS

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# THE QUALITY OF SURFACE WATERS AND GROUND WATERS FROM NEARBY PREAJBA ZONE

Dodocioiu Ana Maria [1], Tudosie A. [2], Buzatu Gilda-Diana [1], Anton Iulia [3]

**Key words***: nutrient losses, liquid runoff, nitrates, fertilizers.*

## ABSTRACT

*The purpose of this work was to study the way how nutrients trained by liquid runff on slope (6%) accumulate in surface water and groundwater in the area bordering Experimental Centre for Pastures Preajba on 6 dates in 2011. Over these waters the following analyses were performed: N - $NH_4$, N - $NO_3$, N - $NO_2$, total N and P total. Following determination we have found that groundwater nutrient values are twice higher for N-$NH_4$, 3-4 times higher for N-$NO_3$, the same in the case of N-$NO_2$ and double for total phosphorus if compared to those from CCCP Preajba, but below the maximum permissible concentration. For Preajba Creek, which runs through this village, the indicators mentioned above were analyzed on 6 dates during 2011 and were below the maximum allowable concentrationfor N-$NH_4$ and N-$NO_3$, but over maximum allowable concentration in the case of N-$NO_2$ and total phosphorus.*

## INTRODUCTION

The eutrophication of surface waters and the contamination of ground waters as a result of massive fertilizer applying has a high impact on ecosystems from many european countries (Ulen, 2005). The Water Frame Directive (W.F.D.) 2000/60/CE assumes an improvement of the quality of surface and ground waters. This fact will determine a dramatic reducing of nutrient losses from farming activity, which will influence the sustainability of agricultural ecosistems on the long run. Additionally, the situation of coastal waters near Baltic Sea, North Sea, etc. represents a special concern for several other european countries. The eutrophycation status of these waters has determined the elaboration of Action Plan which has to be implemented in all european countries (Chardon, 2008).

The Water Frame Directive is considered a progressive and inovative measure that has to improve the surface waters from Europe. It requires evaluations on ecological basis, a wide range of indicators of environment pressures and the ecological stress related with the changing of the reference values. The directive is based on economical realities and the objectives for water bodies are established in function of population needs (Owen, 2011).

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## MATERIAL AND METHOD

The liquid runoff that resulted from surface erosion undergo the following evolution: one part leaches into the soil, another part is retained by down slope and another part is collected in creeks and streams and reaches the surface running waters where they can determine pollution and eutrophication.

In order to analyse how the nutrients are taken away by liquid runoffs on slope in the Preajba nearby zone, there were analysed the waters from the vicinity of the Experimental Centre for Pastures Preajba, District Gorj, as follows: the water from two wells, from the experimental centre and from the village and also the water from two water courses: Preajba creek and Amaradia River; the analyses were made six times a year in 2011. The analyses were: $N - NH_4$, $N - NO_3$, $N - NO_2$, total N and total P.

Determination of nitrites in water has been done by colorimetric method with Griess reagent. Nitrate determination has been done by colorimetric method using phenoldisulfonic acid. Ammonium determination was performed by titration, phosphorus determination was determined colorimetric. Methods used for determining indicators are those developed by ICPA Bucharest.

## RESULTS AND DISCUSSIONS

Analyzing the nutrient content from the well from Experimental centre, there were obtained the following results that are written in the first table. All results are contained in tables 1-4.

Table 1

The nutrients content (nitrogen and phosphorus) from the well located in the Preajba Experimental Centre in 2011 (355 m altitude)

| Indicators | Normal values | Values recorded at: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 05.03 | 03.04 | 11.06 | 20.08 | 18.09 | 16.10 |
| $N-NH_4$, mg/l | 1 | 0.11 | 0.06 | 0.02 | 0.00 | 0.00 | 0.00 |
| $N-NO_3$ mg/l | 50 | 5.20 | 8.4 | 11.2 | 9.6 | 8.4 | 6.2 |
| $N-NO_2$ mg/l | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| Total P mg/l | 0.5 | 0.08 | 0.11 | 0.07 | 0.05 | 0.05 | 0.10 |

There can be observed that the values recorded in this well with all indicators are under normal values, so the water has a very good quality.

The results of indicators from a well located in the village of Preajba, 3 km away from the experimental centre and at an altitude of 170 m, show an increase of the nutrients content from 0.06-0.11 mg/l to 0.07-0.17 mg/l with ammonium indicator, from 5.2-9.6 mg/l to 21.2-28.3 mg/l with nitrates indicator and from 0.05-0.11 mg/l to 0.11—0.21 mg/l with total phosphorus.

This increase of the analyzed indicators and poor quality of the water from this well is due to lower altitude, applied fertilizers yet, especially to population husbandries and unproper deposition of resulting manure and liquid dejections.

The analysis of the main quality indicators on nutrients from running waters from the vicinity of the Experimental Centre of Preajba and Amaradia River in Barbatesti point, aproximatelly 30 km away from the experimental centre is given in the tables below.

Table 2

The nutrients content (nitrogen and phosphorus) from the well located in Preajba village in 2011 (170 m altitude)

| Indicators | Normal values | Values recorded at: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 05.03 | 03.04 | 11.06 | 20.08 | 18.09 | 16.10 |
| $N\text{-}NH_4$. mg/l | 1 | 0.17 | 0.11 | 0.15 | 0.12 | 0.08 | 0.07 |
| $N\text{-}NO_3$ mg/l | 50 | 20.4 | 22.7 | 25.4 | 28.3 | 21.2 | 23.6 |
| $N\text{-}NO_2$ mg/l | 0.01 | 0.0 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 |
| Total P mg/l | 0.5 | 0.21 | 0.18 | 0.20 | 0.16 | 0.11 | 0.17 |

Table 3

The nutrients content (nitrogen and phosphorus) from Preajba creek in 2011

| Indicators | Normal values 161/2006 Document | Values recorded at: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 05.03 | 03.04 | 11.06 | 20.08 | 18.09 | 16.10 |
| $N\text{-}NH_4$. mg/l | 0.4 | 0.38 | 0.34 | 0.30 | 0.30 | 0.28 | 0.23 |
| $N\text{-}NO_3$ mg/l | 1.0 | 1.02 | 0.98 | 0.90 | 0.82 | 0.64 | 0.71 |
| $N\text{-}NO_2$ mg/l | 0.01 | 0.02 | 0.03 | 0.02 | 0.03 | 0.02 | 0.01 |
| Total N mg/l | 1.5 | 1.72 | 1.51 | 1.37 | 1.28 | 1.11 | 1.01 |
| Total P mg/l | 0.015 | 0.017 | 0.011 | 0.018 | 0.022 | 0.016 | 0.015 |

Table 4

The nutrients content (nitrogen and phosphorus) from Amaradia River, Barbatesti point in 2011

| Indicators | Normal values 161/2006 Document | Values recorded at: | | | | | |
|---|---|---|---|---|---|---|---|
| | | 05.03 | 03.04 | 11.06 | 20.08 | 18.09 | 16.10 |
| $N\text{-}NH_4$. mg/l | 0.4 | 0.712 | 0.676 | 0.664 | 1.211 | 0.823 | 0.874 |
| $N\text{-}NO_3$ mg/l | 1.0 | 2.531 | 1.611 | 2.01 | 1.002 | 0.154 | 2.020 |
| $N\text{-}NO_2$ mg/l | 0.01 | 0.041 | 0.028 | 0.072 | 0.028 | 0.025 | 0.028 |
| Total N mg/l | 1.5 | 3.72 | 3.01 | 3.25 | 2.61 | 3.00 | 3.44 |
| Total P mg/l | 0.015 | 0.035 | 0.032 | 0.064 | 0.055 | 0.054 | 0.061 |

In Preajba creek the content of ammoniacal nitrogen is under the normal value established by 161/2006 Document of 0.4 mg/l with all determination data. The same situation is recorded with nitrates indicator. Nevertheless the nitrites indicator is overpassed with all determination data. The content of total nitrogen is overpassed only with the first determination, at 05.03. 2011, the other determinations being under the normal value. The

total phosphorus is overpassed with small values, of 0.01-0.02 mg/l with all determinations due to manure liquid dejections from animals.

Adverselly, in Amaradia River, that collects several tributaries till this point from the hilly area of Gorj County and along with them the slope runoffs from familial husbandries and animal farms, the nutrients load overpasses the normal values with all analyzed indicators at all determination dates and, as a result, the water has a poor quality.

## CONCLUSIONS

The ground waters from Experimental Centre of Preajba located at an altitude of 355 m has had the values of analyzed indicators, $N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NO_2$, much under the admissible limits with all 6 determination dates, sometimes, certain values being 0.00 mg/l which proves that the water is very clean.

In Preajba village, 3 km away from experimental centre, at an altitude of 170 m, the values of nutrients content from ground water is 2 times higher with $N\text{-}NH_4$, 3-4 times higher with $N\text{-}NO_3$ and the same with $N\text{-}NO_2$ and double with the case of total P as compared with the ones from experimental centre yet under MCL (Maximal Concentration Limit). The Preajba creek that crosses through this village has the analyzed indicators $N\text{-}NH_4$ and $N\text{-}NO_3$ under the MCL, yet the $N\text{-}NO_2$ and Pt overpasses the limit with all 6 determination dates.

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# METHODS AND TEHNOLOGIES TO MINIMIZE THE LOSSES OF NUTRIENTS ON THE SLOPE SOILS

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***Key words****: nutrients, soil, fertilization, run off, pollution.*

## ABSTRACT

*In this paper there are presented three methods used to reduce the loss of nutrients on slopes soils, namely: the use of organo-mineral fertilizers with phosphorus, the use of buffer strips and use of some filtration materials for water in streams. After three years of experimentation, it was found that the use of organo-mineral fertilizer has led to the reduction of phosphorus losses from soil, using grassy buffer strips determined a decrease in soil nutrients lost on slopes and the use of some straw bales installed on water courses have contributed to a water purification of nutrients.*

## INTRODUCTION

The movement of the soil particles and the accumulation in areas located on slopes is a factor of the instability of the ecosystem what means the left overs and high quantities of nutrients get to the rivers and this is the effect of the pollution. The rolls of the nutrients in the contents of different types of water was unknown for a long time because of the content of the phosphorus from soil, the industrial activities and the types of used water (Ulen, 2005).

In accordance with COST Action 869 - Final Report (2011), Johannessen (2010) and Chardon (2010), the movement of the phosphorus in the different types of water is affected by the various factors listed below:

- holding psychics-chemical because of the complex of clay from soils;
- mineralization the organic phosphorus from soil;
- processes to control solubility and the movement of the phosphorus in soil;
-the conditions of climate;
- the agriculture processes;
-the type of fertilization with phosphorus used.

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## MATERIAL AND METHOD

To be able to control the losses of the nutrients from soils in slope, we did a series of researches. As examples of the methods and technologies to minimize the losses of the nutrients we used (Foster, 1988):

- the fertilization with phosphorus organic-minerals;
- the used of corridors buffer;
- the filtration material of the water from creeks;

In the years 2009-2011 at the Research Center for the Cultural of Grassland Preajba, the studies were made on the phosphorous and on the type organic-mineral fertilization L120. The type of fertilization organic-mineral with lignite contains clay polymer, formaldehyde polymer and salts of phosphoric and sulfuric acid used at the activation of coil.

For this process were used buffer corridors from natural grassland next to the creek of Preajba. The corridors buffer had the width of 10 meters made from species of: *Agrostis capillaris, Festuca rubra, Cynosurus cristatus, Lollium perenne, Anthoxanthum odoratum, Trifolium repens*.

Also there were used bales of wheat that were put perpendicular on the direction of draining of the Balasan creek.

## RESULTS AND DISCUSSIONS

Fertilization processes with phosphorus organic-mineral.

In L120 fertilizer, nitrogen ions are bound either as ammonium humates or as polimmer amide and as a form of ammonium sulphate salt or ammonium phosphate, phosphorus as ammonium phosphate and potassium ionic (Dorneanu, 2006).

As a result, the nutritive elements appear with different shape, with an organic-mineral mold that is slowing down and lasting the hydrolysis processes, the processes with nitrogen complex, which is the result of losing the necessary phosphorus, in superior calcium phosphorus inaccessible for plants (Passeport, 2010).

Annual were followed the losses from the Preajba soil, based on the precipitations and the types of fertilization used at the scale P162. For the years 2009-2011 the results are presented in table 1.

Table 1

The phosphorus losses from the Preajba soil, based on the culture and fertilization used in 2009-2011

| Variants | Annual precipitations (mm) | Run off ($m^3$/ha) | Eroded soil (t/ha) | Percolated Phosphorus (Kg/ha) |
|---|---|---|---|---|
| Meadow seeded(standard) | 593 | 650,7 | 2,98 | 0,33 |
| Meadow seeded N162P81K80 | 593 | 621,3 | 2,71 | 0,27 |
| Meadow seeded L120 | 593 | 631,4 | 2,82 | 0,20 |
| Maize (standard) | 593 | 733,7 | 5,58 | 0,68 |
| Maize N162P81K80 | 593 | 797,8 | 5,36 | 0,51 |
| Maize L120 | 593 | 727,4 | 5,45 | 0,33 |

Based on all the results following medium quantities of precipitations as example 593 mm/annual, the losses of soil are smaller under the sowing grassland than the corn culture (2, 71-2, 98 t/ha smaller than 5, 36-5,58 t/ha).

The quantities of phosphorus lost in soil were situated between 0, 22-0, 33 kg/ha at the sowing grassland and between 0, 33-0, 68 kg/ha at the corn culture.

As a result the use of organic-mineral fertilization based on lignite minimizes the losses of phosphorus in soil. The value for the sowing grassland was 0,20kg/ha different of the value for 0, 27-0, 33 kg/ha based on using chemical fertilization and 0,33kg/ha different than 0, 51-0, 68 kg/ha for the corn culture.

Using the buffer corridors for reducing the nutrients losses

In the year of 2011 were followed the quantities of soil and nutrients that were lost on the slope soil without buffer corridors next to the Preajba creek and with buffer corridors. The results are presented below, in table 2.

Table 2

The losses of soil and nutrients using the buffer corridors next to the Preajba creek

| Specification | Draining liquids $m^3$/ha | Affected soil t/ha | Humus Kg/ha | N Kg/ha | P Kg/ha | K Kg/ha |
|---|---|---|---|---|---|---|
| Without buffer corridor | 674,2 | 0,78 | 111,2 | 2,94 | 0,114 | 0,59 |
| With buffer corridor | 326,3 | 0,286 | 14,5 | 1,22 | 0,053 | 0,34 |

Based on the results presented in the table below, the conclusions are:

-the value of the draining liquids based on without using the corridors buffer is 674,2 m/ha and only 326,3 m/ha using the corridors buffer with width of 12m.

-the value of the quantities of affected soil is 0,286 t/ha using corridor buffer and 0,780 t/ha without corridor buffer;

-the value of clay losses is 111, 2 kg/ha without corridor buffer and 14, 5 kg/ha for corridor buffer;

-the value of nitrogen losses is 2, 94 without corridor buffer and 1, 22 kg/ha with corridor buffer;

-the value of phosphorous losses is 0,053 kg/ha with corridor buffer and 0,114 kg/ha without corridor buffer;

-the value of potassium losses is 0, 59 kg/ha without corridor buffer and 0,34 kg/ha with corridor buffer.

Using filtration materials for creeks water - bales of straws

For the experiment were used bales of wheat straws that were put perpendicular on the draining direction of Balasan creek. During the process were identified the principal nutrients from the Balasan creek before passing through the bales of wheat straw and after passing through the bales of wheat straw after multiple periods of time. The results are marked below, in table 3.

Table 3

The contents of nutrients from Balasan creek water before and after passing through the filtration of the bales of wheat straw

| Analyzed nutrients | Normal values mg/l | Values before filtration | Determined values after the filtration | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1h | 6h | 24h | 48h | 96h |
| $N\text{-}NH_4$ | 0,4 | 1,82 | 1,85 | 1,61 | 1,03 | 0,56 | 0,38 |
| $N\text{-}NO_3$ | 1,0 | 10,65 | 10,66 | 9,52 | 6,33 | 4,11 | 2,77 |
| $N\text{-}NO_2$ | 0,01 | 0,154 | 0,155 | 0,140 | 0,076 | 0,034 | 0,01 |
| Nt | 1,5 | 8,81 | 8,82 | 8,04 | 5,11 | 2,37 | 1,12 |
| $P\text{-}PO_4$ | 0,1 | 0,119 | 0,119 | 0,114 | 0,080 | 0,060 | 0,04 |
| Pt | 0,015 | 0,21 | 0,22 | 0,13 | 0,06 | 0,02 | 0,01 |
| Ca | 50 | 96,3 | 96,5 | 81,3 | 62,6 | 41,5 | 36,2 |
| Mg | 12 | 29,0 | 29,0 | 27,1 | 21,3 | 15,2 | 10,4 |
| K | 12 | 13,6 | 13,5 | 11,8 | 6,5 | 4,2 | 3,7 |

The conclusion based on the results, using some of the bales of wheat for the filtration of water filled with nutrients has favorable effects for the good qualities of water.

Based on the experiment, after one hour, the contents of nutrients remain unchanged once the water was passing through the bales of wheat: 1,85 mg/l for $N\text{-}NH_4$; 10,66 mg/l for $N\text{-}NO_3$; 0,155 mg/l for $N\text{-}NO_2$; 8,82 mg/l for Nt; 0,119 mg/l for $P\text{-}PO_4$; 0,22 mg/l for Pt; 96,5 mg/l for $Ca^{2+}$; 29,0 mg/l for $Mg^{2+}$ and 13,5 mg/l for $K^{+}$.

Also all the analyzed nutrients, except potassium, had higher values than AMC (acceptable maximum concentration).

Fig.1 Bales of wheat

After 6 hours of passing Balasan creek water through the bales of wheat the content of nutrients begins to lower; example: 1,61 instead 1,85mg/l $N\text{-}NH_4$; 9,52 instead 10,65 mg/l $N\text{-}NO_3$; 0,140 instead 0,155 mg/l $N\text{-}NO_2$; 8,04 instead 8,81 mg/l Nt; 0,114 instead 0,119 mg/l P-PO4; 0,11 instead 0,119 mg/l Pt; 8,31 instead 96,3 mg/l $Ca^{2+}$; 27,1 instead 29,0 mg/l $Mg^{2+}$; 11,3 instead 13,6 mg/l $K^{+}$.

Lowering the content of nutrients from water begins to increase after 24, 48 and 96 hours; the content of nutrients from water is touching the normal value being situated at: 0,38mg $N\text{-}NH_4$/l instead 0,4mg/l normal; 2,77 mg $N\text{-}NO_3$/l instead 1,0 mg/l normal; 0,16 mg $N\text{-}NO_2$/l instead 0,01 mg/l normal; 1,12 mg Nt/l instead 1,5 mg/l normal; 0,04 mg $P\text{-}PO_4$/l instead 0,1 mg/l normal; 0,013 mg Pt/l instead 0,015 mg/l normal; 36,2 mg $Ca^{2+}$/l instead 50 mg/l normal; 10,4 mg $Mg^{2+}$/l instead 12 mg/l normal; 3,7 mg $K^{+}$/l instead 20 mg/l normal;

Using bales of wheat is an important method against pollution for running waters.

## CONCLUSIONS

During three years of experiments, between 2009-2011, the losses of phosphorus on the stable Preajba soil were higher for the corn culture than sowing grassland, on 0,26-0,68 kg/ha instead 0,15-0,33 kg/ha.

Using the organic-mineral fertilization based on lignite L120 was a result to decrease the losses of phosphorus from soil because of the shape of organic-mineral mold for the phosphorus which is rushing the process of replacing and insoluble of phosphorus.

Using the higher concentration of phosphorus (superphosphate) for the sowing grassland the losses of phosphorus were 0, 21-0, 25 kg/ha and using L120 the losses were 0, 15-0, 26 kg/ha.

Using the corridors buffer with 8meters width, next to Preajba creek, had as a result a decrease of lost nutrients for the slope soil from this area. As a result for the area without corridors buffer was lost 0, 78 t/ha soil, 111, 2 kg/ha humus, 2, 99 kg/ha P and 0,59 kg/ha K and for area with corridors buffer, the losses were smaller 0,286 t/ha soil, 14, 5 kg/ha humus, 1,22 kg/ha N, 0,053 kg/ha P and 0,34 kg/ha K.

Using as filtration solution bales of wheat situated on the direction of water had as result a good source of purified water from nutrients which had the value to be reduced between 6 to 10 times.

## ACKNOWLEDGMENTS

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# RESULTS ON THE EVALUATION AND LIMITATION OF ATTACK PRODUCED BY OSTRINIA NUBILALIS Hbn. TO MAIZE CULTIVATED ON SANDY SOILS

Draghici Reta[1]

**Key words:** *hybrid, tolerance, frequency harmful, Trichogramma, chemicals*

## ABSTRACT

*Ostrinia nubilalis (Hübner) can cause serious economic damage to maize crop, depending on many factors (hybrid, climatic conditions, technological measures). In this context, the Research - Development Center for Field Crops on Sandy Soils, Dabuleni been studied in the years 2008 and 2010 attack frequency produced by Ostrinia nubilalis Hbn. in some maize hybrids studied in comparative culture of competition and they have studied various options for limiting the attack. The results underlines very significant reduction in attack frequency produced by Ostinia nubilalis Hbn., up to 13.65%, by launching of 150,000 eggs Trichogramma sp in two halves, compared with the untreated control, which registered a frequency of 40.65 %. Between the experimental maize hybrids have remarked by increased productivity: Olt, Milcov, DK-5143 (9206-10476 kg/ha grain).*

## INTRODUCTION

Integrated protection is a modern concept safener, which seeks tuning and combating populations of pests of plants through harmonious combination of different methods of combat (agronomic, biological, chemical, physical) and natural factors control so harmful to organisms be maintained on at a level that does not cause economic losses of crops. Research conducted in the U.S. by JT Krumm, 2008 highlights the importance cultivating Bt maize, in order to reduce production losses due to attack produced by *Ostrinia nubilalis* Hbn. The same results were obtained in Romania by Dorin Jurca, 2009 showing that average production difference between 4 conventional hybrids vs 4 hybrids genetically modified (MON 810) varied from 479 kg / ha, hybrid DKC 315 (FAO 290 ) to 1009 kg / ha - hybrid DKC 5143 (410 FAO). Preference or refusal pests to certain genotypes of maize leaves are linked to DIMBOUA content, a toxic aglucona that cause stagnation of development and 25% mortality of larvae. Has highlighted a correlation between the concentration of this substance and degree of plant resistance against *Ostrinia nubilalis Hbn*. Romanian hybrids HS - 412, HSF - 1700/70, HS - 370 and inbreed lines RT – 31, ft - 144, T - 146, are relatively resistant to pest attack. Research by Barbulescu et al., 1997, 1999 and Popov C., 2003 established the influence of ecological factors on the evolution of insect, pesting mode, damage and establishing tolerance of maize hybrids,

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depending on the size gallery products *Ostrinia nubilalis Hbn*. Special attention was given to research concerning biological control of
some aspects of *Ostrinia nubilalis Hbn* and chemical combat (Rosca I. and Barbulescu A., 1983). Biological control *Ostrinia* by applying bio-based treatments *Ahuringiemsis Bacillus* concentration of 0.1 to 0.2% decrease population density by 50%. Research conducted at SCA Turda by Muresan Felicia, Mustea D. , 1994 looks like Ostrinia nubilalis Hbn. can be countered by launching trichogramma species (*T. maidis, T. evanescens and T. dendrolini*) and by applying insecticides (Sinoratox 35 EC and Decis 2.5 EC). The results obtained in maize after the launch of *Trichogramma species* can ensure maintain populations of *Ostrinia nubilalis* Hbn. below the economic damage threshold (Bas P. si col., 2008). Biological and food special peculiarities determined maize cultivation in various areas of the world, ranked third in area (130 million hectares) after wheat and rice. In our country maize area cultivated with is about 3 million hectares, 70% is concentrated in the south, which determines finding solutions to stability and maximize production results, considering that in these areas there is the possibility of extending the attack, because the occurrence and the second generation in *Ostrinia nubilalis* Hbn.

In this respect the Research - Development Center for Field Crops on Sandy Soils, Dabuleni have initiated research in 2008 having as objective to evaluate and reduce the attack produced by *Ostrinia nubilalis* Hbn. maize grown on sandy soils.

## MATERIAL AND METHODS

In order to evaluate and limit the attack produced by *Ostrinia nubilalis* Hbn. the maize crop was studied in Research - Development Center for Field Crops on Sandy Soils, Dabuleni, in the years 2008 and 2010, attack frequency this pest, depending on hybrid and some biological and chemical control measures. The research was conducted under conditions of irrigation, on a sandy soil with a humus content of 0.61 to 0.91%, and a $pH_{H2O}$ = 6.8 to 7.1 to 5 maize hybrids experienced in cultures comparative competition and in 5 variants pest control. In this sense to Rapsodia hybrid maize was experienced TRICHOTIM biological product, whose active principle: *Trichogramma sp*. - parasitic wasp belonging to the *Hymenoptera* class, family *Trichogrammatidae*, compared with its untreated and chemical pest control variants.

Were determined water forms of plant, juice cell concentration, attack frequency produced by *Ostrinia nubilalis Hbn*., depending on the hybrid and control method, dimensions galleries and production obtained.

Tolerant hybrids was established according the relationship proposed by Barbulescu Al. and Cosmin O., 1997: The average length of cavity - cavity hybrid minimum length ≤ 50% (mean average cavity length hybrids - the average minimum cavity length hybrids). Research results obtained were interpreted by analysis of variance.

## RESULTS AND DISCUTIONS

Climatic factors significantly influence plant growth and development. The largest effects on production capacity and its quality, their temperature and precipitations, which in their interaction giving relative humidity with crucial role in achieving production.

The analysis of climatic conditions at weather station to R.D.C.F.C.S.S. Dabuleni, (Figure 1), reveals differentiations between the two years of study that influenced significantly the frequency of attack produced by *Ostrinia nubilalis Hbn*. Drought conditions created in 2008, due to increased average monthly temperatures and reduced precipitations registered starting with the second decade of June until the end of august

have led to the stagnation of the growth and development of plants, the inhibition physiological processes of plant and to create favorable conditions unfolding the biological cycle of the pest *Ostrinia nubilalis Hbn*. In 2010, following heavy rains of 166.2 mm, which were registered the end of June, when depositing the eggs of *Ostrinia nubilalis Hbn*., a significant part of the egg has discontinued development cycle, frequency attack is significantly reduced compared to 2008.

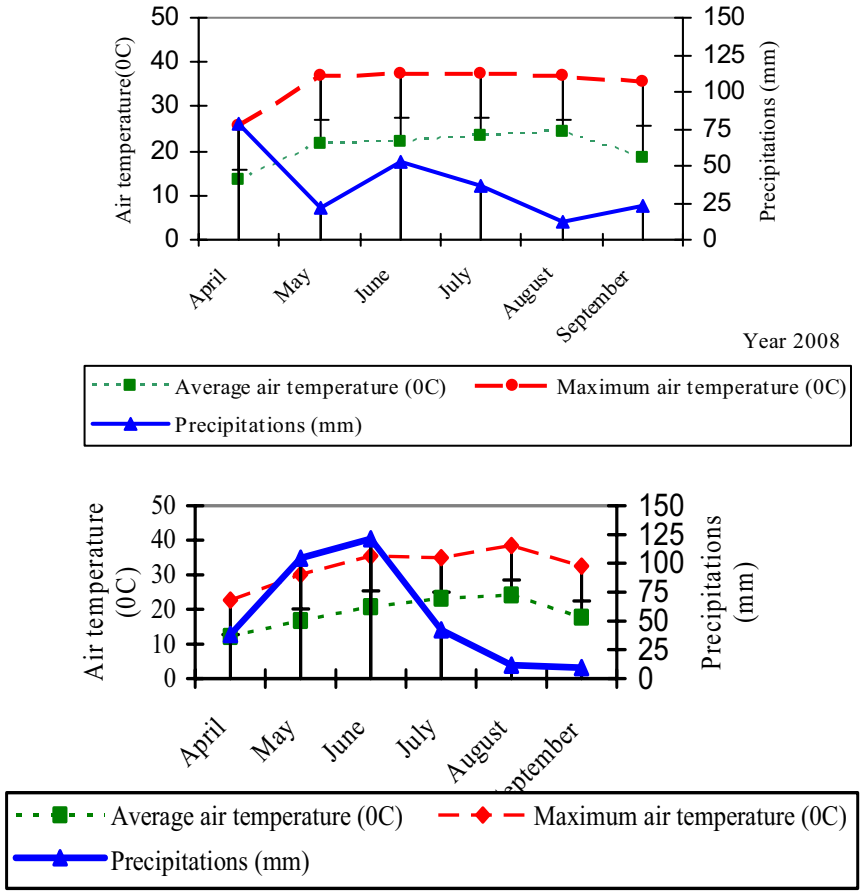


Fig. 1 Climatic conditions of R.D.C.F.C.S.S. Dabuleni meteorological station during the vegetation period of maize

Frequency of attack produced by *Ostrinia nubilalis* Hbn on maize genotypes studied, emphasizes values ranging between 31.2 to 52.1% in 2008 and 11.7 to 18.3% in 2010, according to the biological material used for sowing (Figure .2).

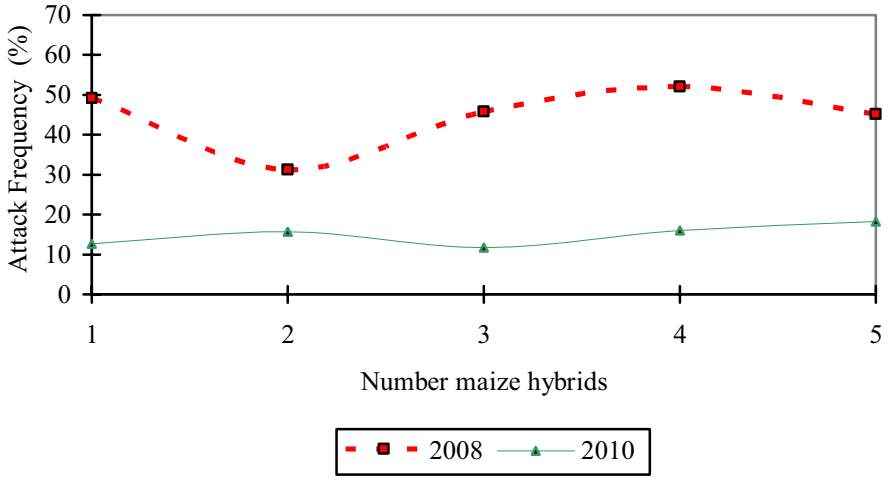


Fig. 2 The attack frequency produced by *Ostrinia nubilalis* Hbn. of maize hybrids studied on sandy soils

Percentage forms of plant water varies significantly depending on the evolution of climatic factors. Thus, unfavorable environmental conditions when plant life activity is reduced than the amount of free water decreases and the bound water increases, which causes a higher resistance of plants to stress factors. In maize leaves free water quantity was influenced by both the variety and climatic factors.

Free water is poorly retained the body and therefore easy circulate, both within the cell and from one cell to another, providing state of turgid cells.

It is the environment in which occuring biochemical processes directly participating in the relevant events. Bound water content oscillated in values ranged between 2.1 -2.8% in 2008 and 1.6 to 2.5 in 2010 (Table 1, Table 2).

As the amount of bound water is higher so that the hybrid is more resistant to adverse weather conditions (high temperatures, drought, low humidity in soil and air). Cellular juice concentration, refractive determined, has the values of 8 to 10.8%, which correlates positively with the percentage of bound water. They highlighted through resistant to drought the following maize hybrids: Generos, Olt, Milcov, DK-5143.

With the relationship established by Barbulescu Al. and Cosmin O.1997 were established tolerance the maize hybrids studied in each of the two years.

As shown in the graphical representation (Fig. 3), in 2008, of the 5 hybrids studied, three hybrids The average length of cavity - cavity hybrid minimum length $\leq$ 50% (50% mean average cavity length hybrids - the average minimum cavity length hybrids)., revealing in this respect hybrids: Rapsodia, Olt, Generos.

In 2010, when conditions were poor for Ostrinia nubilalis Hbn. attack of those five maize hybrids four were tolerant to attack of this pest (Olimpius, Milcov, Olt, DK-5143).

Table 1

Physiological determinations of maize hybrids, phase flourished, 2008

| No. | Maize hybrids | Concentration of cellular juice % | Water forms % | | |
|---|---|---|---|---|---|
| | | | Total water | Free water | Bound water |
| 1 | Rapsodia | 9.8 | 67.58 | 65.32 | 2.26 |
| 2 | Olt | 10.0 | 67.52 | 65.12 | 2.40 |
| 3 | Palatin | 8,5 | 63.44 | 61.34 | 2.10 |
| 4 | Generos | 8,6 | 62.18 | 59.38 | 2.80 |
| 5 | Campion | 9.0 | 65.12 | 63.02 | 2.10 |

Table 2

Physiological determinations of maize hybrids, phase flourished, 2010

| No. | Maize hybrids | Concentration of cellular juice % | Water forms % | | |
|---|---|---|---|---|---|
| | | | Total water | Free water | Bound water |
| 1 | Olimpius | 9 | 68.7 | 66.7 | 2.0 |
| 2 | Milcov | 8 | 70 | 67.5 | 2.5 |
| 3 | Olt | 10.1 | 76.5 | 74 | 2.5 |
| 4 | Danubian | 8.2 | 79.4 | 77.8 | 1.6 |
| 5 | DK-5143 | 10.8 | 74.3 | 71.8 | 2.5 |

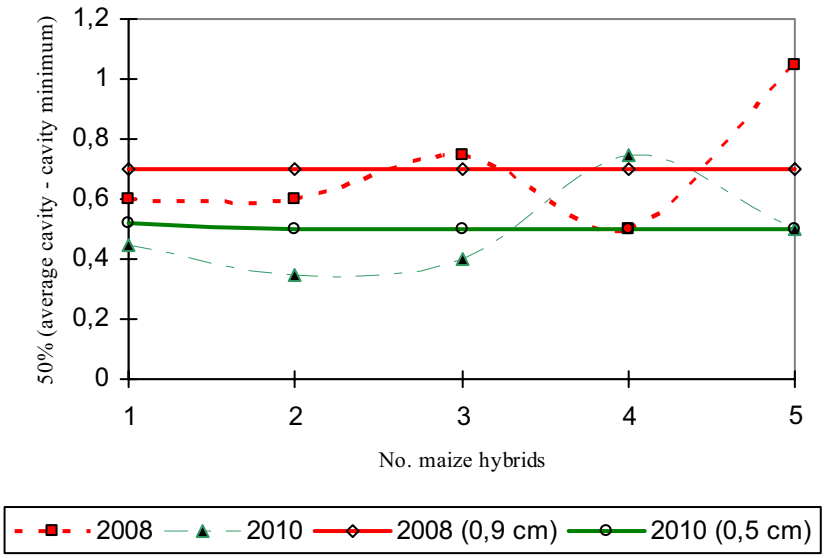


Fig. 3 Tolerance maize hybrids to attack of *Ostrinia nubilalis* Hbn.

Between the experimental maize hybrids have remarked by increased productivity: Olt, Milcov, DK-5143 (9206-10476 kg/ha grain), Table 3.

Table 3

The significance productions experimental maize hybrids sandy soils

| No. | 2008 | | | 2010 | | |
|---|---|---|---|---|---|---|
| | Hybrids | kg/ha | % | Hybrids | kg/ha | % |
| 1 | Rapsodia | 6587 | 97.6 | Olimpius | 7619 $^{0}$ | 84.3 |
| 2 | Olt | 8063 *** | 119.5 | Milcov | 9363 | 103.6 |
| 3 | Palatin | 5999 $^{0}$ | 88.8 | Olt | 10476 * | 116.9 |
| 4 | Generos | 7778 *** | 115.3 | Danubian | 8519 | 94.3 |
| 5 | Campion | 5317 $^{000}$ | 78.8 | DK-5143 | 9206 | 101.9 |
| Media 6748.8 | | | Mt. | Media 9036.6 | | Mt. |
| LSD 5% = 598 kg/ha<br>LSD 1% = 759 kg/ha<br>LSD 0,1% = 997 kg/ha | | | | LSD 5% = 969 kg/ha<br>LSD 1% = 1536 kg/ha<br>LSD 0,1% = 1965 kg/ha | | |

The climatic conditions of the years 2008, 2010, following observations *Ostrinia nubilalis Hbn* adults flight started in the second and third decade of June, and maximum flight was made in decades 1st and 2nd of July, the period when they applied biological and chemical treatments. The first launching of *Trichogramma sp.* was conducted within the period July 5 to 10, and the second launch was performed after 9 days. Environmental conditions influence both the plant, and the pests and entomophagous. By launching

entomophagous *Trichogramma sp*. in system environment- plant-pest-entomophagous, changed the relationship between the population of *Ostrinia nubilalis* and *Trichogramma sp.* in favor of entomophagous, which changed the relationship between pest and plant culture, concretized by reducing the attack and the healthier and larger productions.

Determinations carried out in the launch *Trichogramma sp*., and the application of insecticides Decis 2.5 EC and Calypso 480 CE, shows attack frequency reduction produced by *Ostrinia nubilalis* compared to the untreated control (Table 4).

From the statistical performed after launching of *Trichogramma sp*., frequency attacked plants was reduced significantly distinct, from untreated variant, with 14.35% and after two launchings very significant reduction was 27%.

Compared to biological treatment, chemical treatments had a lower efficacy, in this case attacked frequency of plants are significantly reduced and distinctly significant, compared to the untreated control.

Attack the larvae of *Ostrinia nubilalis Hbn.* first manifested on the upper leaves, then on panicle. After a few days the larvae gnawed leaf sheath and entered the the strain, forming galleries, penetrating then in cob destroying grains.

Production results obtained shows a very significant difference in production of 1727 kg / ha, obtained after performing two launchings with *Trichogramma sp*. 150,000 eggs/ ha, compared with untreated control, (Table 5).

Production results with significantly differentiated as distinct from untreated were obtained in a single launching version of *Trichogramma sp* .. Chemical treatment with

*Calypso 480CE* 0.1 l / ha has contributed to a production differences as statistically significant compared with untreated contro

Table 4.

Biological and chemical treatment effect on attack frequency produced by *Ostrinia nubilalis Hbn.* in maize in 2008 -2010

| No. | Variant | | Attack frequency (%) | Difference from untreated control | Significance |
|---|---|---|---|---|---|
| | Treatment | Dose / ha | | | |
| 1 | Untreated control | - | 40,65 | Mt. | - |
| 2 | Trichogramma – a launching | 150.000 eggs | 26,3 | - 14,35 | 00 |
| 3 | Trichogramma -two launchings | 150.000 eggs | 13,65 | - 27 | 000 |
| 4 | Decis 2,5 EC | 0,3% | 31,45 | - 9,2 | 0 |
| 5 | Calypso 480 CE | 0,1 liter | 28,8 | - 11,85 | 00 |

LSD 5% = 7,2 %;
LSD 1% = 10,3%;
LSD 0,1%= 15,5%

Table 5.

Resultsof production obtained to maize depending on treatment phytosanitary in 2008 - 2010

| No. | Variant | | Plant height -cm- | Cob legth -cm- | Grain Yield | | |
|---|---|---|---|---|---|---|---|
| | Treatment | Dose / ha | | | kg/ha | Difference from untreated control | Significance |
| 1 | Untreated control | - | 176,7 | 18,8 | 6552 | Mt.. | Mt. |
| 2 | Trichogramma – a launching | 150.000 eggs | 183,9 | 20,45 | 7517 | 965 | ** |
| 3 | Trichogramma -two launchings | 150.000 eggs | 188,4 | 21,8 | 8279 | 1717 | *** |
| 4 | Decis 2,5 EC | 0,3% | 183,3 | 19,8 | 6781 | 229 | - |
| 5 | Calypso 480 CE | 0,1 liter | 182,0 | 19,9 | 7223 | 671 | * |

LSD 5% - 649 kg/ha
LSD 1% - 1090 kg/ha
LSD0,1% - 1635 kg/ha

## CONCLUSIONS

In 2008 the pest *Ostrinia nubilalis Hbn.* has found optimal conditions for development of the biological cycle, attacking maize plants with a frequency of 31.2 to 52.1%, depending on the hybrid;

Abundant precipitations of 166.2 mm, which were registered in late June, when depositing the eggs of *Ostrinia nubilalis Hbn*., have reduced the frequency of attacks in 2010 (from 11.7 to 18.3%);

Were highlighted to be tolerant to the attack of *Ostrinia nubilalis Hbn*. hybrids of maize, Olt, Milcov, DK-5143, with production of 9206-10476 kg / ha grain.

Launching of 150,000 eggs Trichogramma sp in two rounds, led to very significant reduction in attack frequency produced by *Ostinia nubilalis Hbn.,* Up to 13.65% compared with untreated control, which was recorded at a frequency of (40.65 %).

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# ANTIBACTERIAL ACTIVITY OF TWO NEW STEROL CARBONATES SYNTHESIZED

Dumitru Elena[1], Chirigiu Liviu[2]*, Bubulică Maria Viorica[2]

***Keywords*****:** *antibacterial activity, diffusion method, sterol carbonates, Staphylococcus aureus, Escherichia coli, Candida albicans*

## ABSTRACT

*In this study was evaluated the antibacterial activity of two new compounds synthesized : 3[4-(phenoxathiin-2-azo)-phenoxy]- cholesteril carbonate and 3[4-(dibenz-p-dioxin-2-azo)-phenoxy]-cholesteril carbonate. The test organisms used were Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922 and Candida albicans ATCC 10231. Comparative inhibitory action was determined by the diffusion method Kirby-Bauer in agar established by National Committee for Clinical Laboratory Standards (NCCLS).The results showed that both compounds have antibacterial activity and antifungal activity.*

## INTRODUCTION

In recent years, were synthesized sterol dimesogens by combining in the same molecule both a structure part specific for nematic state (aromatic Schiff bases) and a structure part specific for cholesteric state (aromatic esters of sterols) (Topală et al., 2003; Topală et al., 2009). This research has gained ground because it was found that sterol mesogens that have in the structure two cycles joined by a group of rigid link (azomethine, azo, ethylene, acetylene) presents a high thermal stability of mesophases.

A series of cholesterol esters with nitrogen and sulfur at sterol C-3 have great interest in medicine, being used to eradicate cancerous tumors, of skin diseases, many such compounds showing strong antimicrobial activity (Manea et al., 2006).

Were synthesized two sterol carbonates with azo structure and heterocycles at sterolic carbon C-3 (figure 1).

Compounds I and II were synthesized based on azo-derivatives 4-(f)phenoxatiin-2-azo)-phenol and 4-(dibenz-p-dioxin-2-azo)-phenol with cholesteril chloroformate, in the presence of pyridine as acid acceptor.

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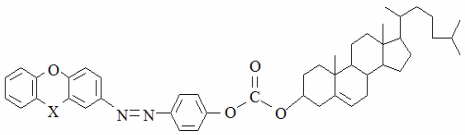

I: X=S

II:X=O

Figure 1. Sterol carbonates: I - 3[4-(phenoxatiin-2-azo)-phenoxy]-cholesterol carbonate; II - 3[4-(dibenz-*p*-dioxin-2-azo)-phenoxy]-cholesterol carbonate

## MATERIAL AND METHODS

To assess the antimicrobial activity of sterol compounds were used the following test organisms: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922 and Candida albicans ATCC 10231.

Comparative inhibitory action was determined by the diffusion method Kirby-Bauer in agar established by National Committee for Clinical Laboratory Standards (NCCLS) (Villanova, 2005) from U.S.A.

This is a common method widely used in laboratories to test a relatively small number of fast-growing bacterial strains, with no significant differences in the growth rate. By submitting discs impregnated with compounds soluble in DMSO on the surface of solid medium seeded with a bacterial culture, antimicrobial active substance will diffuse into the medium having a steady decline of the concentration gradient from the edge of the disc to the periphery.

As culture medium was used agar, distributed in Petri dishes, in uniform layer of 4 mm thickness, pH = 7.2 to 7.4 measured before pouring into plates.

From bacterial cultures were prepared bacterial suspensions 1 / 100 for Staphylococcus aureus and 1/1000 for Escherichia coli. The plates were inoculated using a cotton swab dipped in bacterial suspension. For the adsorption of inoculum, plates were left for 5-8 minutes. Discs were applied on the medium surface using a sterile forceps, then were distributed 200 µl of each dilution made, obtained by dissolving each compound in DMSO.

Incubation of plates was done at 37 $^{o}$C for 17 hours. Bacterial cultures were used for both compounds and standards (represented by Kanamicine for Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 and Naftin for Candida albicans ATCC 10231). Reading was done by eye, measuring the inhibition zone diameter. Expression of results was done by direct transcription of the inhibition zone diameter.

## RESULTS AND DISCUSSIONS

Each sample was tested for three times, and the result represents average value of three inhibition zone diameters of microorganisms' growth test.

In this study was noted a high inhibitory activity of the two compounds on gram-positive bacteria Escherichia coli and Staphylococcus aureus but also an antifungal activity (7 mm diameter of inhibition for compound I and 9 mm diameter of inhibition for compound II ). Results are presented in Table 1, Figure 2.

This antibacterial activity of the two compounds may be due to the fact that they are phenoxathiin derivates and phenoxathiin and a number of its derivatives are known to

have bacterial activity (Behalo, 2010; Beteringhe et al., 2006), anti-inflammatory activity (Hillebrand et al., 1999) and antifungal activity (Meltzer et al., 2001).

Table 1

Antibacterial activity of compounds I-II

| Sample | | 1. Bacteria (diameter of inhibition zone [mm]) | | |
|---|---|---|---|---|
| | | Escherichia coli ATCC 25922 | Staphylococcus aureus ATCC25923 | Candida albicans ATCC 10231 |
| II | | 20 | 19 | 7 |
| I | | 21 | 20 | 9 |
| Antibiotic | Kanamicin | 23 | 22 | - |
| | Naftin | - | - | 16 |

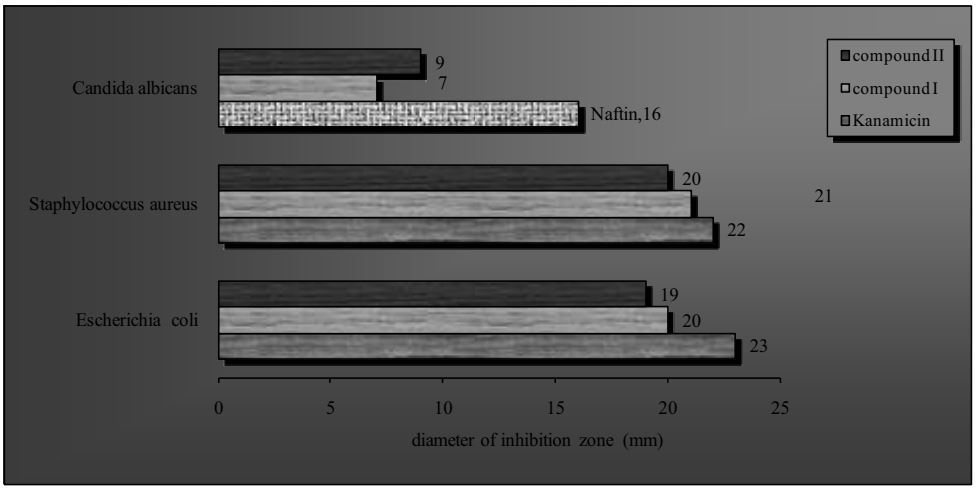


Fig. 2. Antibacterial activity of carbonates I-II

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**Vol. XVII ( LIII ) - 2012**

# TESTING THE ACTION OF NEW SYNTHETIZED CHOLESTEROL DERIVATES ON PLANT CELL

Dumitru Elena[1], Chirigiu Liviu[2]*, Bubulică Maria Viorica[2]

**Keywords:** *cholesterol derivates, genotoxic potential, cytotoxicity.*

## ABSTRACT

*In this study was evaluated the action of 13 new compounds synthesized (five cholesterol butyrate, four cholesterol carbonates and four cholesterol carbamates) on plant cell. Were evaluated macroscopic and microscopic certain aspects related with possible cytogenity and cytotoxicity of this new compounds. Biological material on which research was conducted were embryonic roots of wheat (Triticum variety). The results showed that at least in the case of a plant cell (wheat in this case), used compounds would have a genotoxic potential and can be used with caution at preparation of pharmaceutical forms.*

## INTRODUCTION

A series of cholesterol esters with nitrogen and sulfur at sterol C-3 have great interest in medicine, being used to eradicate cancerous tumors, of skin diseases, many such compounds showing strong antimicrobial activity (Topală et al., 2003; Topală et al., 2009; Meltzer et al., 2001).

Were synthesized cholesterol derivates with heterocycles at sterolic carbon C-3 (table 1, figure 1).

The action of the new synthesized cholesterol derivates on plant cell was assessed. Were evaluated certain aspects related with possible cytogenity and cytotoxicity of compounds from table 1. It has been used for this purpose phytobiological method – Testum Triticum of prof. Grigore Constantinescu (Constantinescu et al., 1963). The experiment took place from 23 to 28 March 2011.

## MATERIALS AND METHODS

Since cholesterol derivatives are insoluble in water, they were solubilised in chloroform, solution was evaporated to dryness, and the obtained residue was used to observe root elongation and mitosis. Biological material on which research was conducted were embryonic roots of wheat (Triticum vulgare Mill, variety Dropia, Gramineae). Seeds were selected uniform, were moistened with distilled water, then were placed for 24 hours at 22 ˚ C in a container. 14 Petri dishes were prepared (13 for samples and one for control)

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with diameter of 20 cm. On the bottom of Petri dishes rings of filter paper were placed and were soaked in distilled water. An equal number of wheat grains was distributed to each recipient (5 caryopsis) and were left to germinate until the main root length reached 0.7 to 1 cm. When the main root length reaches 1 cm, are inserted 5-6 caryopsis germinated in a Petri dish, diameter 10 cm, containing 5 ml test solution and kept at room temperature. The results are expressed compared with blank sample; distilled water was used instead of researched solutions.

Microscopic examination followed changes induced in root tips of *Triticum Vulgare*. Wheat germ roots were sectioned at approximately 5 mm distance on top and stained with dilute acetic orceina as La Cour process. Microscopic examination was conducted on a MBLB 2050 Kruss Binocular Microscope.

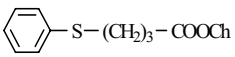
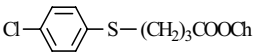
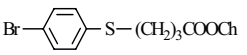
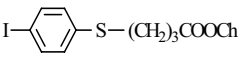
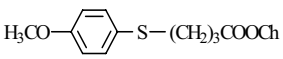
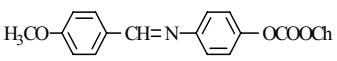
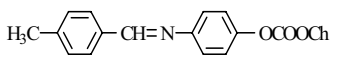
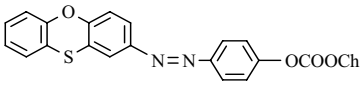
Growth in length evaluation was performed using linear measurements technique, from the baseline of treatment, every 24 hours, over 5 consecutive days this period representing the most active growth phase.

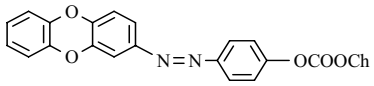
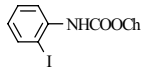
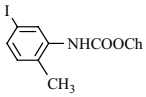
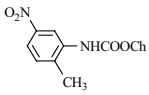
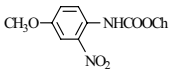
During the 5 days of testing, wheat plants were maintained in a room at a temperature of about 22 ° C and continuous illumination of 2500 lx.

There were made observations on morphological changes (epicotyl appearance and length, aspect and length radicels) (Bodor et al., 1983; Anghel, 1979).

Table 1

Cholesterol derivates used in experiment

| Symbol text | Compound |
|---|---|
| $P_1$ |  |
| $P_2$ |  |
| $P_3$ |  |
| $P_4$ |  |
| $P_5$ |  |
| $P_6$ |  |
| $P_7$ |  |
| $P_8$ |  |

| | |
|---|---|
| $P_9$ |  |
| $P_{10}$ |  |
| $P_{11}$ |  |
| $P_{12}$ |  |
| $P_{13}$ |  |
| where Ch is the cholesterol radical | |

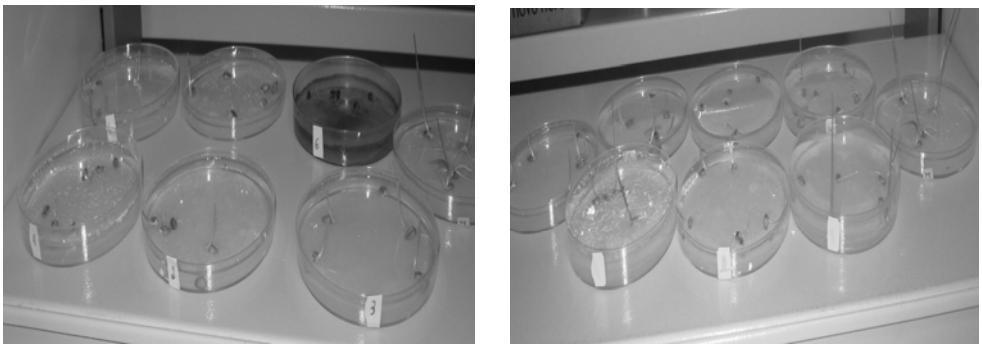
## RESULTS AND DISCUSSIONS

Averages of results obtained by measuring the length of the main roots for 5 consecutive days are presented in figure 1 and table 2.

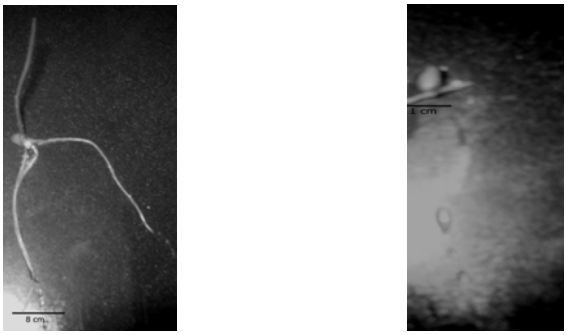
Table 2

Averages of roots elongation for samples 1 to 13 and blank

| No. | Sample | Average of main roots elongation (cm) | | | | |
|---|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h | 120 h |
| 1. | Blank | 1,56 | 3,40 | 4,22 | 6,22 | 6,70 |
| 2. | $P_1$ | 1,22 | 1,60 | 1,82 | 2,22 | 2,36 |
| 3. | $P_2$ | 1,26 | 1,50 | 1,72 | 2,00 | 2,08 |
| 4. | $P_3$ | 1,32 | 1,82 | 2,06 | 2,66 | 2,84 |
| 5. | $P_4$ | 1,30 | 1,38 | 1,64 | 1,86 | 2,00 |
| 6. | $P_5$ | 1,14 | 1,28 | 1,40 | 1,48 | 1,66 |
| 7. | $P_6$ | 0,92 | 0,92 | 0,92 | 0,92 | 0,92 |
| 8. | $P_7$ | 1,12 | 1,68 | 1,74 | 2,14 | 2,26 |
| 9. | $P_8$ | 1,26 | 1,70 | 1,98 | 2,64 | 2,90 |
| 10. | $P_9$ | 1,20 | 1,24 | 1,32 | 1,42 | 1,48 |
| 11. | $P_{10}$ | 1,34 | 1,58 | 1,68 | 2,14 | 2,44 |
| 12. | $P_{11}$ | 1,24 | 1,44 | 1,54 | 1,74 | 1,80 |
| 13. | $P_{12}$ | 1,06 | 1,12 | 1,18 | 1,18 | 1,18 |
| 14. | $P_{13}$ | 1,16 | 1,32 | 1,36 | 1,58 | 1,72 |



a b



c d

Fig. 1. a - samples 1-6 and blank sample after 120 h; b – samples 7-13 and blank sample after 120 h; c – wheat seed from blank sample after 120 h; d – wheat seed from sample 13 after 120 h

In table 3 are presented macroscopic observations on roots of wheat kept for 120 hours in the solutions tested. Chromosomal aberration test (chromosomal fragments, bridges, micronucleus, and other types of structural chromosomal abnormalities) revealed a moderate genetic clastogenic effect worthy of consideration.

On impact with various stressors agents (chemical, physical, biological), these processes can be more or less disturbed, either in terms of inhibition, or in that of stimulation, is register a wide range of effects that influence, in good or bad, cell metabolism processes (Yi and Meng, 2003; Ioniță and Dinu, 2004). Phytobiological testing of the new substances has been imposed of the necessity to evaluate certain aspects related with possible cytogenity and cytotoxicity of compounds.

From the tables and photos presented and by comparison with blank sample, it observes that all solutions of new synthesized compounds inhibit with a certain percentage, increase in length of main root. Thus for the derivate colesteril six (4-(4-methoxybenzylidene)-amino phenoxy carbonate-cholesterol) is observed a total inhibition of root elongation, while for the derivatives 3 and 8 is observed inhibition of primary root by approximately 68 percent of the average of roots blank.

Table 3

Macroscopic observations on wheat plants maintained in tested solutions for 120 hours

| No. | Sample | Macroscopic observations |
|---|---|---|
| 1. | Blank | - normal root growth, no swelling, white, green epycotil of 6 cm; |
| 2. | $P_1$ | - main root growth inhibited, two weak secondary roots, epycotil green; |
| 3. | $P_2$ | - main root growth inhibited, two weak secondary roots underdeveloped with slight brown spots; |
| 4. | $P_3$ | - primary root growth is inhibited by about 60% , twisted secondary roots, whitecolour, solution is slightly opalescent, epycotil is green; |
| 5. | $P_4$ | - white primary root, developed, 3 – 4 secondary roots, white, developed, slightly opalescent solution, green epycotil; |
| 6. | $P_5$ | - primary root is slightly inhibited, the 4 secondary roots are developed, solution is slightly opalescent; |
| 7. | $P_6$ | - totally inhibited growth, opalescent solution; |
| 8. | $P_7$ | - main root is less inhibited, the 4 secondary roots are developed and twisted, white, solution is slightly opalescent; |
| 9. | $P_8$ | - inhibited main root, white, poorly developed secondary roots, twisted; |
| 10. | $P_9$ | - the main root is less inhibited, three secondary roots are white, developed and twisted, the solution is slightly opalescent; |
| 11. | $P_{10}$ | - inhibited main root, white, poorly developed secondary roots, twisted; |
| 12. | $P_{11}$ | - the main root is less inhibited, the 3 secondary roots are developed and twisted, the solution is slightly opalescent; |
| 13. | $P_{12}$ | - the main root growth is inhibited, two weak secondary roots, both the main root and secondary roots have brown spots; |
| 14. | $P_{13}$ | - the main root growth is inhibited total, primary and secondary roots have brown spots, the solution is opalescent; |

The results presented in the tables 2 and 3 are able to make us accept that at least in the case of a plant cell (wheat in this case), used compounds would have a genotoxic potential and can be used with caution at preparation of pharmaceutical forms.

Microscopic examination showed numerous cells with abnormal division phases, nuclei with 1-2 hypertrophied nucleoli, often 2 nucleoli of unequal sizes.

## CONCLUSIONS

Macroscopic observations showed strong inhibition for compounds $P_3$, $P_6$, $P_8$, $P_9$ and $P_{12}$. Changes on cariokinetic film showed cells with dehydrated aspect, nuclei with different shapes. Moderate genotoxic effect clearly indicates cytotoxic action of this type of compounds. Future studies showing possible cytostatic action should be made in future.

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**Vol. XVII ( LIII ) - 2012**

# THE INFLUENCE OF MINING INDUSTRY ON THE WATER QUALITY OF THE UPPER JIU RIVER

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Ciupeanu Călugăru Daniela Eleonora[1]

***Keywords*:** *water pollution, mining industry, physicochemical indicators, water quality*

## ABSTRACT

The water quality in the upper Jiu valley is affected by pollution from mining and coal preparation plants and also by nutrients and a water acidification trend.

This study aims to identify the sources of pollution and to determine the water quality of the Upper Jiu River. The physicochemical analyzes conducted in 2010-2012 revealed values of the suspensions content higher than the MPC (maximum permissible concentrations) in force due to the coal preparation activity. The analyses results showed that the physicochemical indicators of water are below the allowed MPC (excluding the suspensions).

## INTRODUCTION

The water quality is influenced by the environmental conditions: lithology of the catchment, vegetation and climate (Tolgyessy, 2003). The water pollution problem in small catchment basins is more increased than in large catchment basins. The major water quality problems have been identified to depend on the type of pollution (organic, inorganic, with suspensions, biogenic substances etc.) (Meybeck and Helmer, 2009).

The Jiu River is formed of two major tributaries: West Jiu considered as spring, which has its origin in the Retezatul Mic Mountain and has a length of 51 km; and East Jiu which springs from the Şureanu Massif and has a length of 28 km. Their tributaries are limited in length and surface by the catchment area (Savin, 2008).

From all the affected environmental factors the water is the most polluted by direct discharges or by carrying various elements stored in open-pit mines, waste dumps and coal preparation plants (Ostopowich, 2010).

The activity in the Jiu Valley is based on the mining industry and related activities, which depend mostly on the quality and quantity of extracted coal, on the efficient extraction and on the use of this raw material.

This paper aims to determine the pollution factors and how they affect the water quality of the Upper Jiu River.

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The importance of this paper consists in the determination of the point source pollution and the methods to reduce it.

## MATERIAL AND METHOD

For the water quality analysis we started the study from the contaminated water discharge points into the environment due to the mining activity in this area (Fig. 1). The potential hazards produced by the waste water discharges are: contamination of drinking water sources due to the waste water which enters the soil until the groundwater surface; the direct impact on the health of the local population, flora and fauna degradation from the surface waters and facilitation of the development of parasitic microorganisms (Dunca and Berov, 2006).

In the 2010-2012 period there were collected water samples from the two tributaries of the Upper Jiu River, determining the following indicators: particulate matters (gravimetrically determined), pH (determined with Hanna pH meter), nitrates, sulphates, chlorides, ammonium, nitrites, phosphorus, iron, manganese (determined with the DR2010 spectrophotometer), bicarbonates, hardness, COD-Cr (determined with the digital titrimeter), DO (determined by the Winckler method).

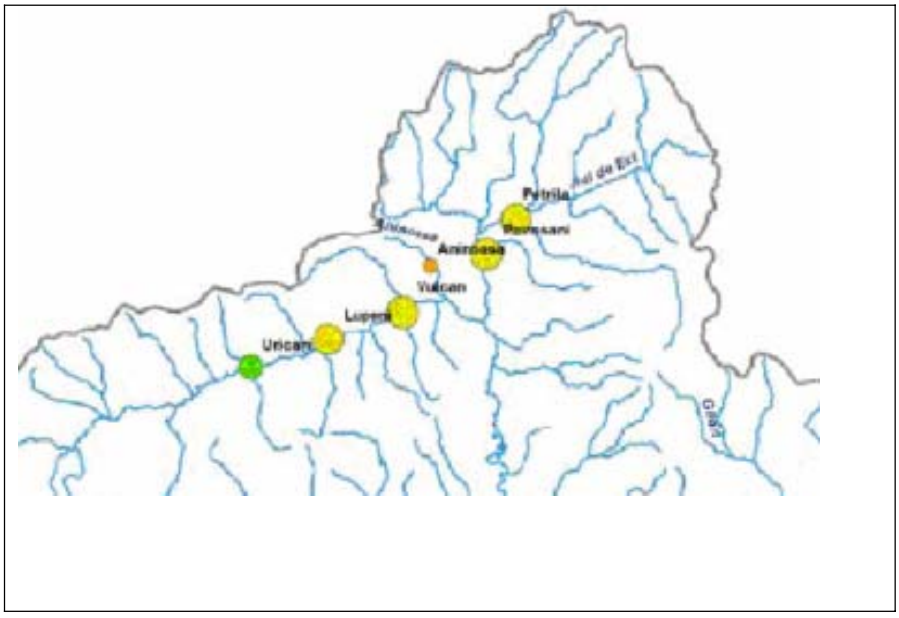


Fig. 1. Mining in the Upper Jiu sub-basin

## RESULTS AND DISCUSSIONS

Some of the most important environmental pollutants in the upper Jiu basin are mining industry and coal preparation plants.

Mining is responsible for a high degree of water pollution with metal ions, solid mineral suspensions, organic matters and nutrients (Eckenfelder and others, 2009).

The mining industry with the specific activities determines a significant environmental impact by loading the surface waters with contaminated elements, changing the hydrology, and by groundwater pollution (Ionică, 2002).

Uricani, Vulcan, Lupeni coal mines have as main activity the pitcoal extraction, affecting the water quality by increasing the concentration of particulate matters.

SC Termoelectrica SA Paroşeni has as main activity the production of electricity and heat, the water used for cooling is discharged in West Jiu.

Paroşeni coal mine has the main activity of executing the opening, preparation and exploitation of pitcoal. In this area there are also discharged the domestic waste waters that require purification.

Coroeşti coal preparation plant is located on West Jiu (Vulcan town) and its activity is the prewash of superior and inferior coal. Also there are discharged domestic waste waters in the Jiu River. This is the only plant with tailings management facility and a water treatment plant, the water being recirculated.

Lonea, Petrila coal mines and Petrila coal preparation plant are sources of pollution for East Jiu. Petrosani town discharges chemically contaminated waste water from SC UPSRUEEM SA and SC UNIROM SA plants. These are chromium and cyanide waste water that are treated, and some waters are recirculated through installations.

There are also discharged in Jiu the waste water from hospital, domestic waste water, waste water discharged from PECO gas stations, which have a high content of oils and fats. The analyses of the water of Jiu which crosses the areas with industrial activity rank the river in the 3rd water category (Drăghici, 2006).

The main results of the coal mines are presented as follows (Fig. 2-5).

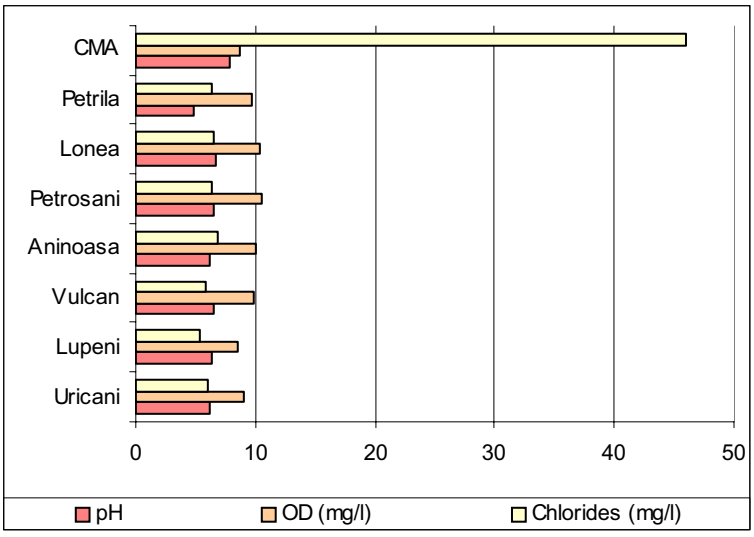


Fig. 2. The average pH values, dissolved oxygen and chlorides, in 2010-2012

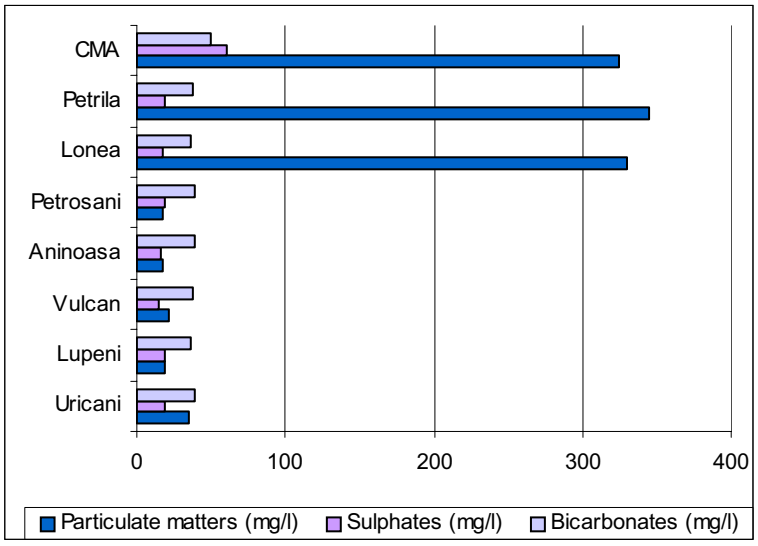


Fig. 3. The average particulate matters values, sulphates and bicarbonates, in 2010-2012

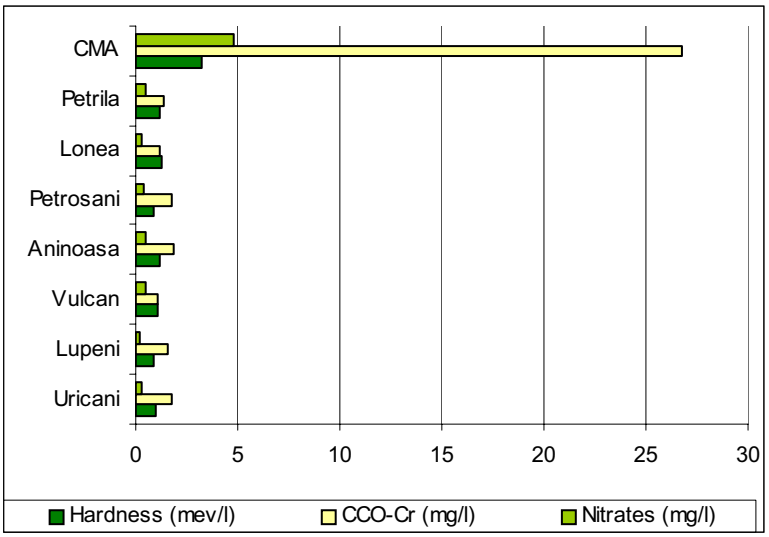


Fig. 4. The average hardness values, chemical oxygen demand and nitrates, in 2010-2012

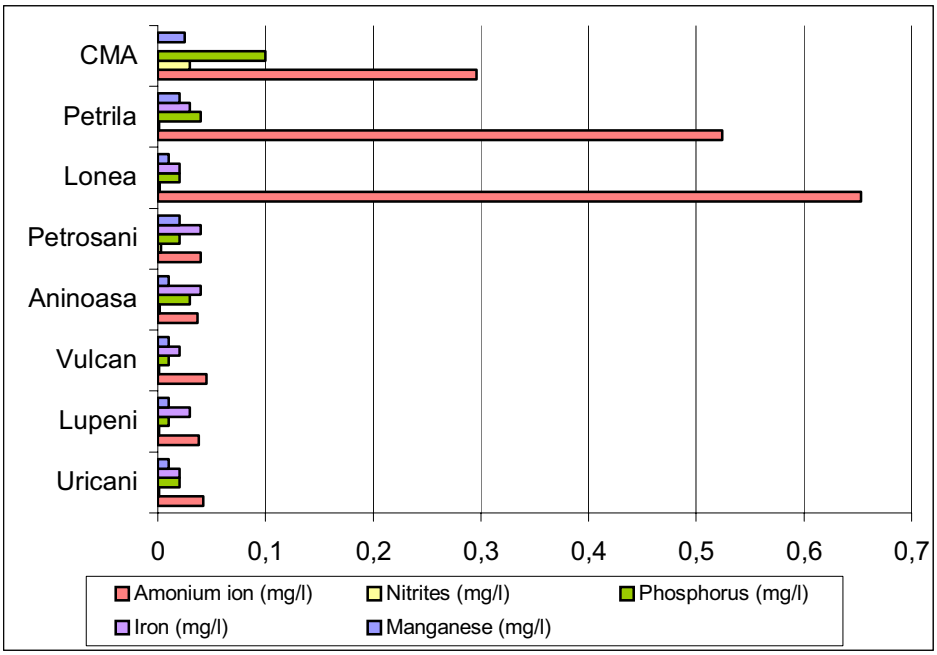


Fig. 5. The average ammonium ion values, nitrites, phosphorus, iron and manganese, in 2010-2012

The analyses have shown that East Jiu is particularly polluted due to large quantities of solid suspensions.

Petrosani has an increased pollution due to the location at the confluence of the two tributaries of Jiu. In this area, the Jiu water is brown due to the clay-coal suspensions, leading to the disappearance of flora and fauna of the area. Its water is improper for agricultural and industrial uses downstream (Drăghici, 2006).

Due to the high level of pollution, the Jiu river water does not have self-cleaning capacity. The Jiu River is not included in the fishery fund and mountain tourism.

## CONCLUSIONS

The results of the analyzes conducted in 2011-2012 showed that the pollutants of the Upper Jiu River are within the MPC allowed by current standards, with the exception of particulate matters, ammonium ion and dissolved oxygen.

The main sources of Jiu water pollution are the coal underground extraction and coal processing.

The Upper Jiu river waters can be classified in the 2$^{nd}$ category of quality, and in Petroşani area they are classified within the 3$^{rd}$ category.

The water quality has been improved with the modernization of Coroeşti Coal Preparation Plant (the only one in operation), the new coal preparation technology implies the waste water total recirculation and the usage of rinse reagents.

The Jiu river waters have a high content of nutrients due to agricultural activities, phosphate content with technological origin.

The surface water pH values are slightly acid, which is correlated with the increased organic load of water.

West Jiu is more polluted than East Jiu, due to the more polluting industrial activities.

To reduce the negative environmental impact it is necessary that the polluting enterprises to be equipped with treatment plants and to be modernized with processing technological processes.

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# THE INFLUENCE OF GEOMETRICAL, EMISSION AND METEOROLOGICAL FACTORS ON POLLUTANTS DISPERSION IN ATMOSPHERE

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Militaru Ileana[2]

***KEY WORDS:*** *atmospheric pollutants*, *dispersion models, atmospheric stability, emission parameters*

## ABSTRACT

*It was studied the influence of geometrical factor (the height of source), emission rate and atmospheric stability on the pollutant concentration for a plane source, using the environmental program SCREEN 3. In the same time, the emission parameter was correlated with the maxim concentration of pollutant in atmosphere*

## INTRODUCTION

The explosive development of human activities, especially in industry, agriculture, tourism and urbanization domain, proves that the man intervention on the environmental conditions has exceeded the thresholds of the nature regeneration. The sources of pollution are multiple and can be classified according to different criteria: the nature of pollutants (natural, industrial, agricultural), toxicity, size sources (point, linear, plane) (Hâncu & Marin, 2008). Serious is that the local pollutants action not only in the emission zone, but are transported in space, air, surface water, soil and groundwater. It is very important to act on pollution at source by adopting clean technologies, environmentally friendly, ensuring socio-economic sustainable development without environmental damage. But once produced, both current pollution cases (if it is not reduced to the maximum allowable), and especially in cases of accidents, (whose risk should be reduced as much), the pollutants should be monitored, must be known the laws by they propagate and should be identified and act for defence or to suppress their negative effects, if is possible (Hâncu and al., 2003)

The pollutant dispersion in the atmosphere is influenced by type (stable, neutral, unstable) and emission parameters (geometrical characteristics of the source, location, emission rate).The boundary between stable and unstable atmosphere is given by the adiabatic thermal gradient curve. When the slope of external thermal gradient curve is greater than the adiabatic thermal gradient ($\gamma < \gamma_e$), $\gamma_e$ is called superadiabatic and the atmosphere is unstable. When the two curves have equal slopes, the atmosphere is neutral; when the slope of external thermal gradient is less than the slope of adiabatic thermal

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gradient ($\gamma_e > \gamma$), $\gamma_e$ it is called *underadiabatic* and the atmosphere is stable. In a layer where the air temperature is constant $\gamma e = 0$ (isothermal atmosphere), the atmosphere is stable (Anghel & Giosanu, 2010).

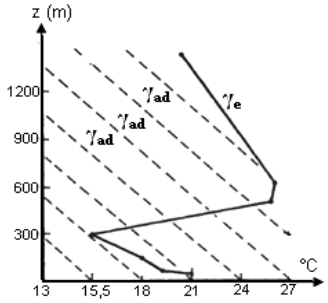


Fig. 1. The thermal inversion for industrialized cities (Barry and Chorley, 2003)

Some configurations of relief favour the appearance of thermal inversion; in this case, the pollutants produced at the surface of the earth no longer disperse in the atmosphere (Danielson and al., 2003). Another type of inversion is the "radiation" and is produced by the radiation emitted by the earth's surface at night; the cold air, like pollutants produced during the night, are "trapped" under layers of warmer air from above (process called inversion cap) (Blake and al., 2008). Figure 1 presents a different thermal inversion (called the press inversion); it occurs in industrialized cities (where large masses of warm air are at the heights of 200-300 m), which keeps the cooler air on the soil, including pollutants contained there in.

The atmospheric stability can be recognized by the form of gas (containing smoke or visible vapor); it's called flare of smoke or steam. In the superadiabatic atmosphere (unstable), the shape is like a loop and produce high concentrations of pollutants where touches the ground; in the neutral atmosphere (stable), the shape is conical and it disperses both vertically and horizontally during propagation. In a stable atmosphere, with thermal inversion, the flare is disperses in a thin layer, at constant level. In unstable atmosphere, with thermal inversion in superior layers, it appears the phenomenon called fumigation (the pollutant flare is captured in layers of warm air and maintained long enough time in contact with the ground), situations which can generate hazardous pollution.(Iribarne & Godson, 2002). The cross section of concentration in the pollutant flare is a Gaussian curve; the pollutant distribution is described by the standard deviation of the distribution curve, on the horizontal and vertical directions (figure 2).

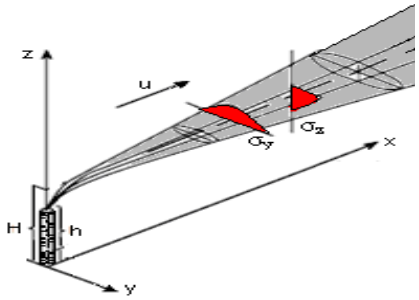


Fig. 2. The cross section of pollutant concentration (Mason & Hughes, 2001)

To estimate the concentration of air pollutants emitted by different sources are using dispersion models. The most frequently used is the Gaussian model (Gryning & Schiermeir, 2000). Such models are important for the governmental agencies responsible for the protection and management of ambient air quality. Models are typically used to determine whether the existing industrial facilities are in compliance with national ambient air quality. Moreover, they serve to help to design effective control strategies to reduce emissions of harmful air pollutants. The Gaussian dispersion models are available for short periods of estimating (the order of minutes or hours), when the atmospheric situation does not change much. This model has a number of limitations applicable: are not valid for wind speeds below 1 m/s or distances exceeding 50 km, analyzes the pollutants not reacting chemically and not disregard the gravitational sedimentation of particles with sizes larger than 10 μm. There are more complex programs that include these situations.

## MATERIAL AND METHODS

In this paper we study only the dispersion models of pollutants emitted from a single source, type area. It is necessary to introduce data as: weather conditions (speed and direction of wind, stability class, ambient air temperature and the presence of any thermal inversions), emission parameters (type and source location, height, stack diameter, exit velocity and temperature gas) and location. Some of the regulatory model recommended by the U.S. Environmental Protection Agency includes the use of environmental programs: SCREEN3 and AERMOD. We used this software (SCREEN3) to study the influence of various factors (geometrical, weather, etc.) on the position and value of maximum pollutant concentration for a surface/area source.

## RESULTS AND DISCUSSION

The stack height of source plays an important role in the dispersion of pollutants in the atmosphere; if the stack height is greater, the pollutant removes from the soil and disperses into the atmosphere. A dispersion of pollutants over a big area reduces their concentration, thus respecting the limits imposed by environmental legislation in force.

A) The influence of stack height

To study the influence of geometrical parameters on concentrations of pollutants emitted by a source of type area (surface), it used the SCREEN program, imposing the following conditions: the source is in a rural area, we choose one single class of stability (3) for a distance no longer than 500m from stack. The height stack is between 10 m and 50 m.

Table 1

The influence of height stack on the variation of pollutant concentration

| $c_{max}$ ($\mu g/m^3$) | Position $c_{max}$ d(m) | Stability class | Emission rate (g/s) | Height stack (m) | Downwash phenomenon |
|---|---|---|---|---|---|
| 1399 | 94 | 3 | 2,5 | 10 | No |
| 1190 | 147 | 3 | 2,5 | 15 | No |
| 648 | 201 | 3 | 2,5 | 20 | No |
| 405 | 257 | 3 | 2,5 | 25 | No |
| 276,4 | 314 | 3 | 2,5 | 30 | No |
| 199,8 | 372 | 3 | 2,5 | 35 | No |
| 150,9 | 430 | 3 | 2,5 | 40 | No |
| 117,8 | 488 | 3 | 2,5 | 45 | No |
| 92 | 494 | 3 | 2,5 | 50 | No |

From table 1 it remarks that the maxim values of pollutant concentration depends by the height stack; the point of maxim pollutant concentration is situated at a greater distance from the source, as the stack height is greater. In none of the studied cases previously not appeared downwash effect.

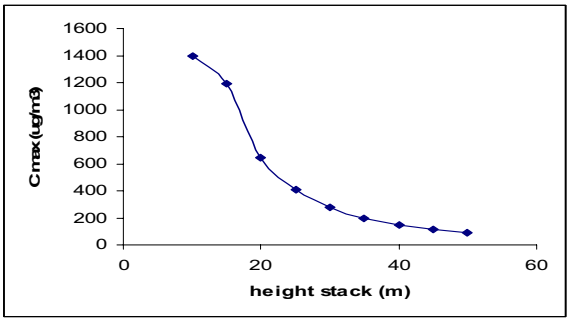


Fig. 3. $C_{max}$ versus height stack

From figure 3 we notice a decrease in pollutant concentration with increasing stack height. It is preferable that the stack height exceeds the height of obstacles around the source, thus avoiding the appearance of downwash effect produced by large buildings and structures.

B) The influence of emission rates

Thereafter, it was studied the variation of the maximum concentration depending on the emission rate of pollution. The parameters as: the class of atmospheric stability (3), the height stack (10 m) were constant. The obtained data after it used the SCREEN program are in table 2.

Table 2.

The influence of emission rate on the variation of pollutant concentration

| $c_{max}(\mu g/m^3)$ | position $c_{max}$ d(m) | Stability class | Emission rate (g/s) | H coş (m) | Downwash |
|---|---|---|---|---|---|
| 1323,5 | 94 | 3 | 1 | 10 | No |
| 2798 | 94 | 3 | 2 | 10 | No |
| 4198 | 94 | 3 | 3 | 10 | No |
| 5597 | 94 | 3 | 4 | 10 | No |
| 6996 | 94 | 3 | 5 | 10 | No |
| 8395 | 94 | 3 | 6 | 10 | No |

From the table it can be seen that the distance at which Cmax of the pollutant occurs remains constant, regardless of the emission rate. The downwash phenomenon didn't appear.

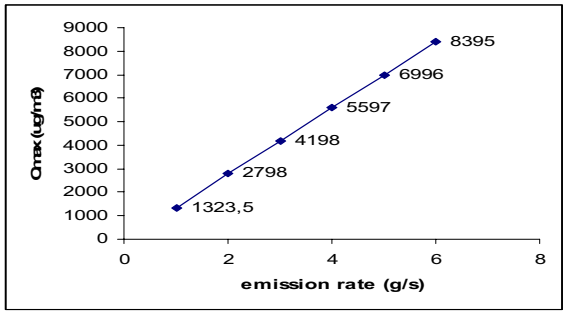


Fig. 4. $C_{max}$ pollutant versus emission rate

Figure 4 shows that with increasing emission rate, the maximum concentration of the pollutant increases; Cmax position to source remains constant (94 m), for the same stability class (3) and the same height of the stack (10m).

C) The influence of meteorological factors (atmospheric stability class)

For studying the influence of atmospheric stability on pollutant concentrations, the Screen program run for atmospheric stability classes 1 to 6; the results are in table 3. The emission rate (2.5 g/s) and the height stack (10m) were constant.

Table 3.

The influence of atmospheric stability class on the variation of pollutant concentration

| $c_{max}(\mu g/m^3)$ | position $c_{max}$ d(m) | Stability class | Emission rate (g/s) | Height stack (m) | Downwash |
|---|---|---|---|---|---|
| 365 | 125 | 1 | 2,5 | 10 | No |
| 348 | 186 | 2 | 2,5 | 10 | No |
| 333 | 322 | 3 | 2,5 | 10 | No |
| 312 | 488 | 4 | 2,5 | 10 | No |
| 306 | 488 | 5 | 2,5 | 10 | No |
| 253 | 488 | 6 | 2,5 | 10 | No |

The downwash effect doesn't appear.

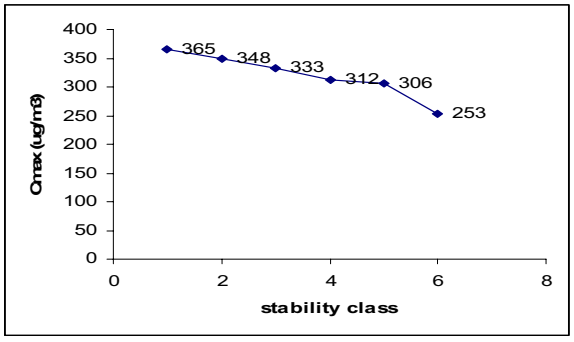


Fig. 5. $C_{max}$ pollutant versus stability class

It remarks from Figure 5 that the maximum pollutant concentration value corresponding with a great atmospheric stability (class 1), while a strong instability (class 6) will lead to a strong dispersion, so a minimum concentration of pollutant (253µg/mc)

## CONCLUSIONS

The study highlights the importance of knowing the laws dispersion of pollutants in the atmosphere and the factors that influence it (stack height, emission rates, and meteorological stability class). It remarks that:

The pollutant concentration decreases with the increasing of stack height. It is preferable that the stack height is greater than the buildings around the source because, by reflection on them, the airflow formed turbulence and a depression near the obstacle, so the wedge pollutant goes down.

The concentration of the pollutant increases with the increasing of emission rate; under certain conditions, the maximum concentration can occur at the same distance from the source.

The atmospheric instability causes a stronger dispersion of the pollutant.

Further research is required to study the cumulative dispersion of air pollutants (for more sources) by analyzing izoconcentration curves.

For industrial stacks, the reducing of the amount of pollutants emitted into the atmosphere can be achieved by taking into account the type of source and nature of pollutants emitted from the activities carried out, by attaching specific filters and by imposing regulations on the operation in stationary regime for the sources with high pollution.

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# THE DYNAMICS OF THE CABABID POPULATIONS (CARABIDAE, COLEOPTERA) IN THE BEECH FOREST FROM BUILA-VÂNTURARIŢA NATIONAL PARK

Huidu Magdalena [1]

***Keywords***: *carabid populations, dynamics, beech forest*

## ABSTRACT

*From April till October of 2007 I have taken quantitative samples of the carabid populations from two sites (F1 and F2) located in the beech forest from Bistriţa Gorges, which belongs to the Buila-Vânturariţa National Park. Within the two forests sites I have identified 28 carabid species belonging to 10 genera, 11 species were common in both sites.*

*The studied populations were characterized by a small number of (eu)dominant species: 3 eudominant and 2 dominant carabid species in F1, representing 20% of the total number of species identified here and 3 eudominant carabid species and 2 dominant ones in F2, representing 35.73% of the total number of species.*

*The species diversity of the carabid populations from the two sites from the beech forest varied widely throughout the year, influenced by the abiotic factors.*

## INTRODUCTION

The Buila-Vânturariţa National Park is also a site of the European Ecologic Network Nature 2000 and was found in 2004. Buila-Vânturariţa is the smallest national park in Romania, with a total area of 4500 ha. It is located in the Vâlcea County, in the South-Western part of Romania and in the southern corner of Căpăţânii Mountains.

The Park comprises the linear limestone ridge of the Buila-Vânturariţa Massive, 14 km long. Bistriţa Gorges are one of the four gorges sectors from the park, located in the south-weastern part of the park.

There are only few ecological studies of carabids in forest ecosystems in Buila-Vânturariţa National Park.

The ground beetles are used as boindicators in studies of the habitats conservation or deterioration status and for environmental predictions concerning the ecosystems tendency (Niţu 2007). Carabids are all known as extremely sensitive to abiotic and biotic factors; they respond quickly to habitat alteration and can be easily collected using pitfall traps (Avgin & Luff 2010). Carabids are excellent subject for studies of the effects of

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fragmentation on species with different dispersal abilities and habitat requirements (den Boer 1990, Niemelä et al. 1993).

The breeding period is an important factor affecting the survival of carabid populations, especially in the forests from the temperate zones. Species that breed during the time when the forest management practices are very intense (spring), are affected more that the ones that breed during periods of less anthropic disturbance (autumn) (Magura et al. 2000). Although forest management affects specialized forest species, the dominance structure does not necessarily change.

The present study represents a contribution to the knowledge of the carabid populations annual dynamics in two sites from the beech forest located in Bistriţa Gorges.

## MATERIALS AND METHODS

The fauna material was taken on a monthly base during April-October 2007 in two locations (F1 and F2) from a beech forest in Bistriţa Gorges (Buila-Vânturariţa National Park).

In the beech forest, the dominant species – *Fagus sylvatica* is accompanied by many *Sambucus nigra* shrubs, and the herbaceous layer is mainly formed of *Asplenium scolopendrium*, *Salvia glutinosa* and *Polygonatum latifolium*. The beech forest is 100 years old. Unlike F2, the F1 site is located at the forest border. The type of soil is litosol. The two studied sites were not affected by forestry.

In order to collect the carabid fauna, I used pitfall traps (plastic cups of 450 ml and with a 10 cm diameter) filled with a 4% formalin solution. The captured fauna was placed in tubes with 70% alcohol. In order to be weighed, the fauna was partly dried out with filter paper.

Within each site of the studied habitat were placed 9 traps (meaning 81 traps in each habitat type during all the period of the study). The distance between two traps was about three meters.

The collected carabid fauna was determined up to the species level using the identification keys (Trautner & Geigenmüller 1986, Hůrka 1996, Lindroth 1974).

To characterize the dynamics of the carabid populations, I have determined the number of individuals for each identified species, the sex structure, the relative abundance, (in order to establish the numerical dominance), the Shannon-Wiener index of diversity. Every individual of every species was weighed separately. I also determined the mean individual biomass (MIB) by dividing the total biomass of a species to the number of individuals of that species.

Knowing that the carabids distribution depends on several abiotic factors such as temperature, humidity, food resources presence and distribution of competitors (Lövei & Sunderland 1996), the correlation coefficient "r" was calculated between the carabids numerical abundance and the values registered for temperature and humidity. The obtained values were tested for significance using Student Test ($\alpha = 0.05$).

## RESULTS AND DISCUSSIONS

Numerical abundance, sex structure and species relative abundance

Of the total 28 captured species in the two sampling sites, 11 of them were common species. The collected carabid fauna counted 786 individuals (560 individuals were captured in F1 site and 226 in F2 site). In F1 sampling area, there were identified 25 carabid species, and in F2 only 14 species (Table 1).

Table 1

The numerical abundances (no.ind.), relative abundances (rel.ab.%) and the sex structure in the two sampling sites from the beech forest (F1 and F2)

| SPECIES | F1 | | | | F2 | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of. ind. | | | Rel. ab% | No. of. indiv. | | | Rel. ab% |
| | total | males | females | | total | males | females | |
| *Abax ater (Abax parallelepipedus)* (Piller & Mitterpacher 1783) | 159 | 66 | 93 | 28.4 | 84 | 38 | 46 | 37.3 |
| *Abax parallelus* (Duftschmid 1812) | 11 | 6 | 5 | 1.96 | 22 | 12 | 10 | 9.78 |
| *Amara aenea* (De Geer 1774) | 1 | | 1 | 0.18 | | | | |
| *Amara familiaris* (Duftschmid 1812) | | | | | 1 | 1 | | 0.44 |
| *Amara montivaga* Sturm 1825 | 1 | | 1 | 0.18 | | | | |
| *Carabus (Autocarabus) cancellatus* Illiger 1798 | 1 | | 1 | 0.18 | | | | |
| *Carabus (Chaetocarabus) intricatus* Linnaeus 1761 | 1 | | 1 | 0.18 | | | | |
| *Carabus (Eucarabus) ullrichi* Germar, 1824 | 4 | 1 | 3 | 0.71 | | | | |
| *Carabus (Eucarabus) arvensis (C. arcensis)* Herbst 1784 | 134 | 63 | 71 | 23.9 | 9 | 6 | 3 | 3.56 |
| *Carabus (Megodontus) violaceus* Linnaeus, 1758 | 37 | 13 | 24 | 6.61 | 25 | 22 | 3 | 23.6 |
| *Carabus (Oreocarabus) glabratus* Paykull 1790 | 20 | 6 | 14 | 3.57 | | | | |
| *Carabus granulatus* Linnaeus 1758 | 44 | 6 | 38 | 7.86 | 4 | 1 | 3 | 1.78 |
| *Carabus (Procrustes) coriaceus* Linnaeus 1758 | 72 | 28 | 44 | 12.9 | 25 | 12 | 13 | 11.1 |
| *Carabus (Tomocarabus) convexus* Fabricius 1775 | 18 | 4 | 14 | 3.21 | 3 | 1 | 2 | 1.33 |
| *Cychrus caraboides* (Linnaeus 1758) | | | | | 3 | | 3 | 1.33 |
| *Cychrus semigranosus* Palliardi 1825 | 4 | 2 | 2 | 0.71 | 3 | 2 | 1 | 1.33 |
| *Hapalus latus* (Linnaeus 1758) | 1 | 1 | | 0.18 | | | | |
| *Harpalus affinis (H. aeneus)* (Schrank 1781) | 6 | 1 | 5 | 1.07 | 2 | 1 | 1 | 0.89 |
| *Harpalus laevipes* Zetterstedt 1828 | 2 | 2 | | 0.36 | 3 | 3 | | 1.33 |
| *Harpalus tardus* (Panzer 1796) | 1 | 1 | | 0.18 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Leistus (Pogonophorus) rufomarginatus* (Duftschmid 1812) | 1 | 1 | | 0.18 | | | | |
| *Molops piceus* (Panzer 1793) | 10 | 5 | 5 | 1.79 | 13 | 5 | 8 | 5.78 |
| *Nebria (Boreonebria) rufescens (Stroem, 1768* | | | | | 1 | | 1 | 0.44 |
| *Pterostichus (Bothriopterus) oblongopunctatus* (Fabricius, 1787) | 15 | 9 | 6 | 2.68 | | | | |
| *Pterostichus (Haptoderus) unctulatus* (Duftschmid 1812) | 5 | 1 | 4 | 0.89 | | | | |
| *Pterostichus (Platysma) niger* (Schaller 1783) | 7 | 4 | 3 | 1.25 | | | | |
| *Trechus quadristriatus* (Schrank 1781) | 2 | 1 | 1 | 0.36 | | | | |
| *Trechus rubens* (Fabricius 1792) | 3 | | 3 | 0.54 | | | | |
| TOTAL | 560 | 221 | 339 | 100 | 226 | 104 | 122 | 100 |

Analyzing the dominance structure, it can be noticed that the studied populations were characterized by a small number of (eu)dominant species: 3 eudominant carabid species (*Abax parallelepipedus, Carabus arvensis, Carabus coriaceus*) and 2 dominant species (*Carabus violaceus, Carabus granulatus*) in the case of the carabids captured in F1. The (eu)dominant species identified in F1 site represented 20% of the total number of species captured here. In F2 site were 3 eudominant carabid species (*Abax parallelepipedus, Carabus violaceus, Carabus coriaceus*) and 2 dominant (*Abax parallelus, Molops piceus*) those representing 35.73% of the total number of species. The presence of 5 (eu)dominant carabid species in F1 site and 5 (eu)dominant species in F2, leads to the assumption that those species are less sensitive to variations of abiotic factors (humidity and temperature) (Table 1).

Among the (eu)dominant species identified in the carabid populations structure from F1 and F2, three of them were common: *Carabus coriaceus, Carabus violaceus, Abax parallelepipedus*.

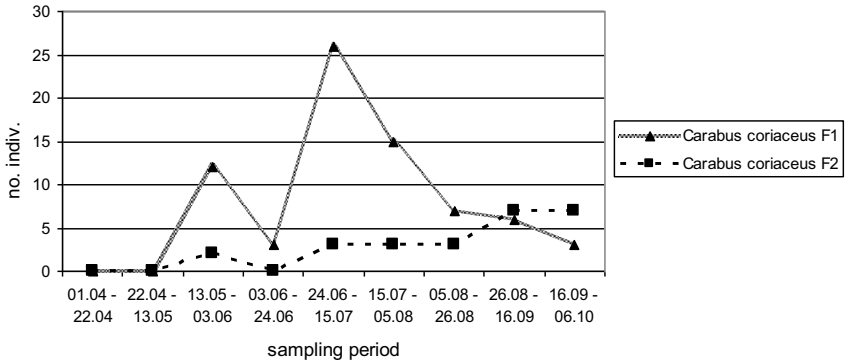


Fig. 1. The annual dynamics of *Carabus coriaceus* during 2007

Analyzing the annual dynamics of *Carabus coriaceus* numerical abundance in F1 and F2 sites, it can be noticed a similar variation in both forest sites, although the highest numerical abundance was registered in F1 forest (from April till September, with a minimum point in June). That may be due to the abiotic (temperature, humidity) and biotic (food resources) factors that limit the development of this species in F2 site (Fig. 1).

A similar situation was observed also for *Abax parallelepipedus* (Fig. 2).

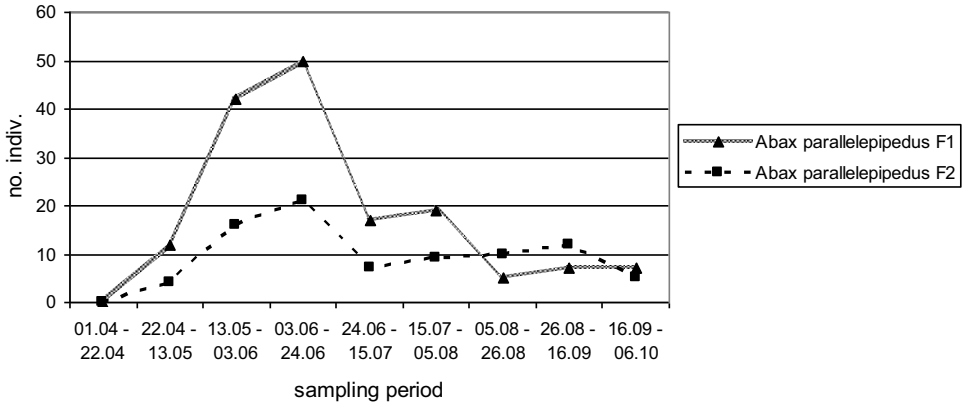


Fig. 2. The annual dynamics of *Abax parallelepipedus* during 2007

For *Carabus violaceus*, the dynamics of the numerical abundance registered in F1 and F2 sampling locations was almost identical during June-July period. During autumn, although the variation was similar in F1 and F2 sites, the numerical abundance values observed in F2 forest were higher (Fig. 3). That was probably due to the richer nutritional resources and to the abiotic factors which had a positive influence on carabids development in F2. It also be noticed that the life cycle begins in April in F1location, and later in F2.

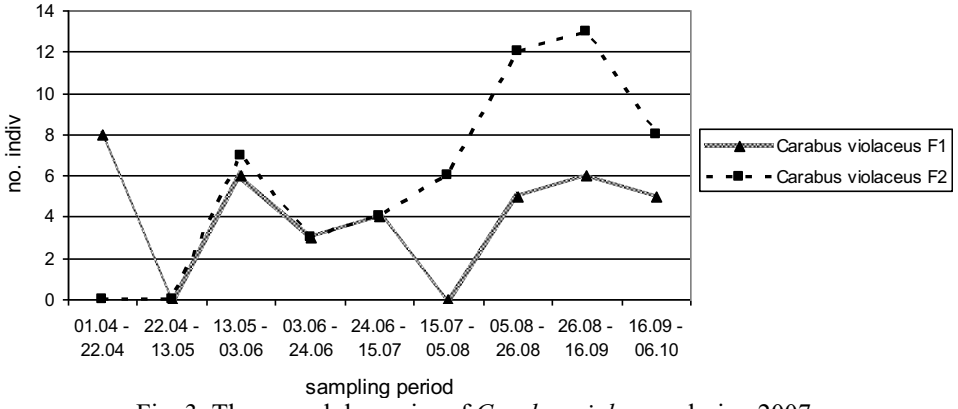


Fig. 3. The annual dynamics of *Carabus violaceus* during 2007

Dynamics of the carabids sex structure

With respect to the carabids sex structure, it can be observed that the number of females was higher then the number of males captured in both sampling sites (Table 1). The

carabid females represented 60.54% of the total captured carabids in F1 site. In F2, the females represented 53.99%.

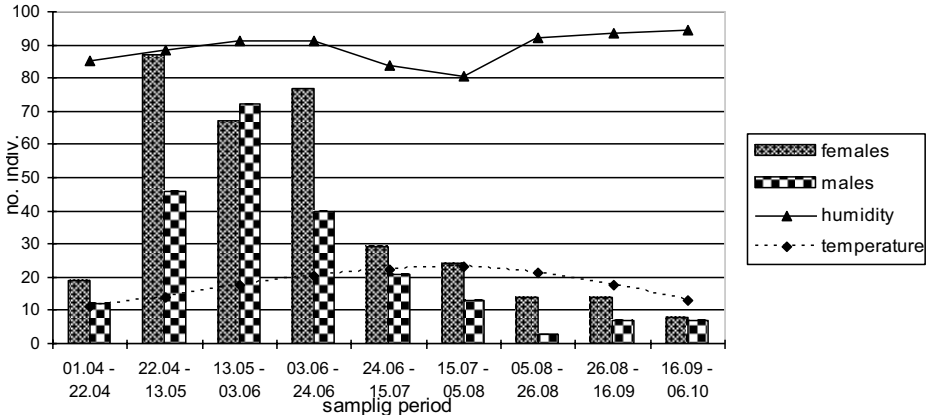


Fig. 4. The dynamics of the carabids sex structure in F1during 2007

Analyzing the dynamics of the carabid populations sex structure in the case of the carabids identified in F1 site, it can be noticed a high number of females during spring and early summer (April-June) (Fig. 4), suggesting that the most numerous species were the spring breeder ones. This fact is also confirmed by the populations structure according to the breeding period (Table 2). The facts that the predominant species were the spring breeders and that the high numerical abundance was observed during April-June period (both in females and males), can be explained knowing that females, after laid their eggs, entered deeper into the soil avoiding the severe climatic conditions, and the males died.

A similar situation was observed in the case of the carabids identified in F2 sampling site (a high numerical abundance for females and males during spring and early summer and a high percentage of spring breading species) (Fig. 5). Nevertheless in the case of the carabid populations identified in F2 site, a high number of individuals was captured also in August-September.

Table 2

The structure of the carabid populations (%) from the beech forest (F1 and F2) according to the breeding period of the species

| Breeding period | F1 % | F2 % |
|---|---|---|
| Autumn breeders | 28 | 28.57 |
| Spring breeders | 60 | 50 |
| Summer breeders | 4 | - |
| Spring-summer breeders | 4 | 14.28 |
| Variable | 4 | 7.14 |

A common characteristic for the two carabid populations was that most of the species were spring breeders (Table 2). That leads to the assumptions that the beech forest, in both cases, provides enough food for the adults and larvae survive.

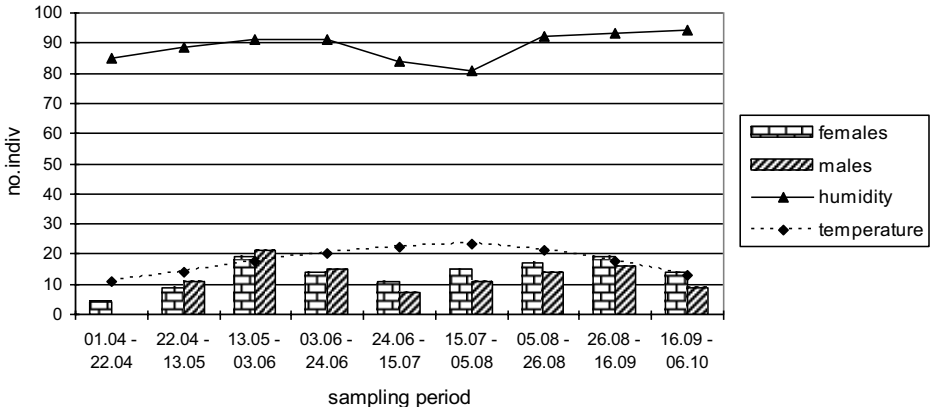


Fig. 5. The dynamics of the carabids sex structure in F2 during 2007

In both cases (F1 and F2) the number of carabids registered a peak during spring (both females and males) and decrease during summer. The lower numerical abundance observed during summer, was due to the influence of the abiotic factors (humidity decrease and temperature increase); it is well known that the carabids activity is influences by the variation of the climatic factors (Honěk 1997, Varvara 2009). A similar situation was also observed in a mixed tree forest from the Buila-Vânturariţa National Park (Huidu 2010).

Analyzing the values of the correlation coefficient calculated between the abiotic factors (humidity and temperature) values and the numerical abundance of the carabids identified in F1 site, it can be noticed that there was no significant correlation between them. An opposite situation was observed for the carabids captured in F2 site. Here, there was a positive correlations statistically significant ($r = 0.522$ for humidity, $r = 0.475$ for temperature, $\alpha = 0.05$) among the abiotic factors and the numerical abundance. It can be concluded that the temperature and the humidity facilitated the carabids development in F2 location. During 2007, for the studied areas, the temperature varied from a monthly mean value of $10.9^0$C to $23.27^0$C. Also, the monthly mean values for humidity varied from 80.75 mm to 94.25 mm.

Dynamics of the carabids mean individual biomass (MIB)

Analyzing the variations of the MIB during sampling period, it was noticed that, in both investigated carabid populations, the highest value was registered in spring and early summer (May-June). The MIB dynamics was similar to the one of the numeric abundance of the species: a peak observed in May-June and a slow decrease during summer. In the case of the carabid populations identified in F2 site, unlike the ones captured in F1, I noticed a new increase in MIB values during autumn (Fig. 6).

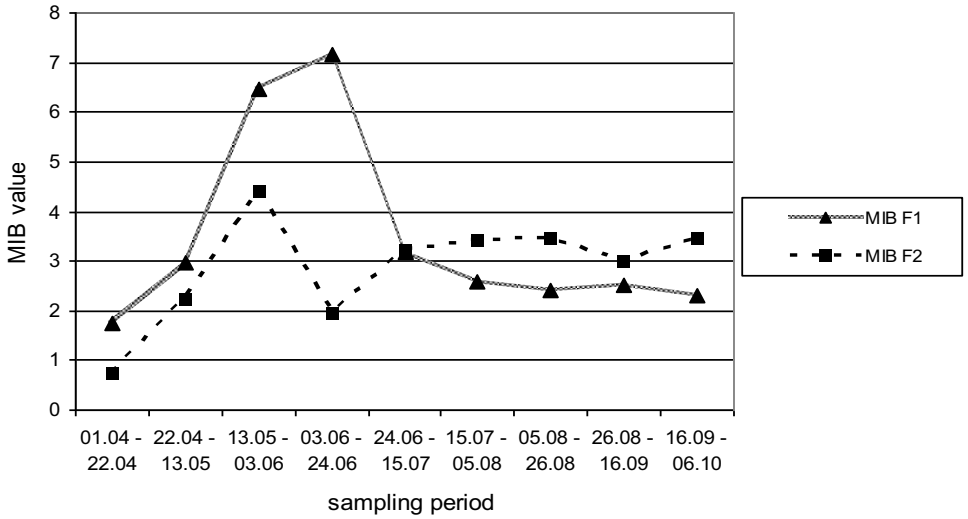


Fig. 6. The dynamics of the MIB of the carabid populations (F1 and F2) during sampling period

The total MIB value calculated in the case of the carabids captured in F1 site was higher then the total MIB of the ground beetles identified in F2 sampling site. This can be explained by the higher number of species and the higher numerical abundance observed in F1 forest. The presence of a high number of species and a high numerical abundance in F1 forest can be explained by the fact that the studied area is located at the forest border, near a meadow, many of the species identified here coming from the meadow, especially during summer, when the humidity registered a decrease (Niţu 2007).

During spring, the greatest contribution to the MIB value was brought by the eudominat species *Carabus arvensis* (59.53% of the total MIB of the carabids captured in April-May) for the carabids identified in F1. In the case of the carabids captured in F2 sampling site, the highest contribution to the MIB value was brought by the eudominant species *Carabus violaceus* and *Abax parallelepipedus*. For the summer period, the maximum contribution to the MIB value was brought by *Carabus coriaceus* in the case of the carabid populations from F1, and *Abax parallelepipedus* for F2. In autumn, the (eu)dominant species *Carabus coriaceus* and *Carabus violaceus* had the greatest contribution to the MIB values for both carabid populations.

Analyzing the annual dynamics for the common (eu)dominant species, in the case of *Abax parallelepipedus* it was noticed a peak registered in August in F1. The highest value for F2 was observed in July-August, when the MIB values for F1 forest registered a decrease (Fig. 7).

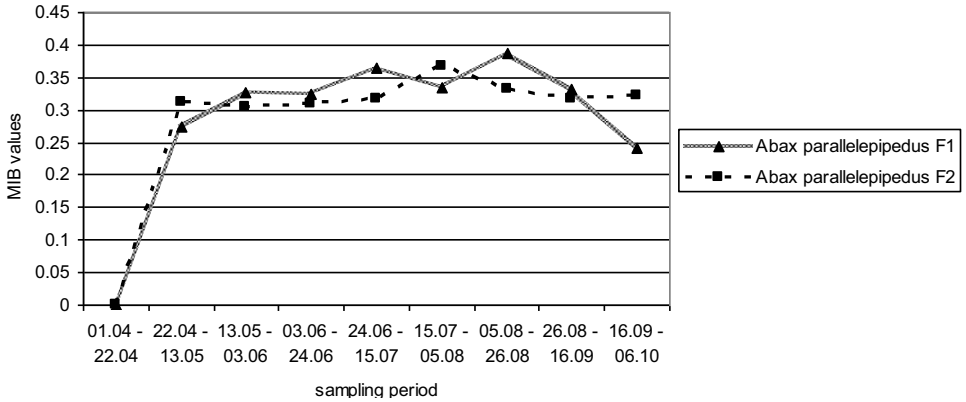


Fig. 7. The dynamics of *Abax parallelepipedus* MIB during 2007

For *Carabus coriaceus,* the species with the highest contribution to the MIB values in both forests, the annual dynamics showed a similar variation pattern with the one observed for *Abax parallelepipedus* in the case of the carabid population captured in F1. In F2 forest, *Carabus coriaceus* registered a peak for the MIB values during April-May. This indicated high food resource in the habitat in this period. The lowest values for MIB were noticed in July-August (Fig. 8).

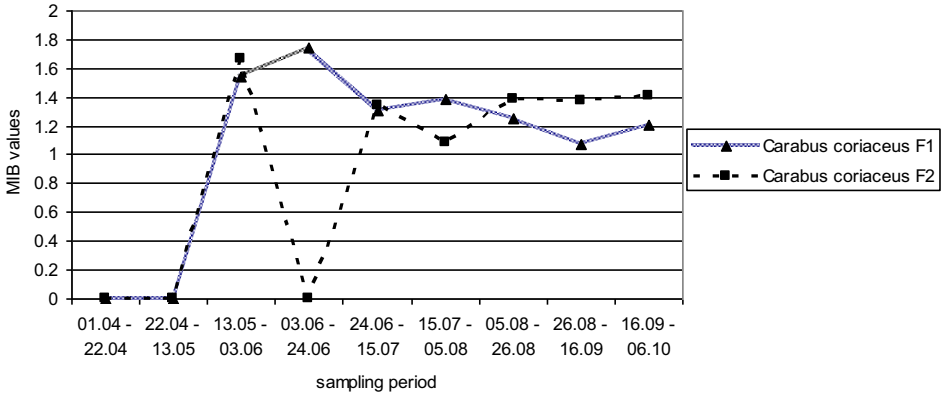


Fig. 8. The dynamics of *Carabus coriaceus* MIB during 2007

In the case of *Carabus violaceus*, the annual dynamics of MIB in F1 forest indicated a peak in June-July period, while the highest value for F2 was observed during August (Fig. 9).

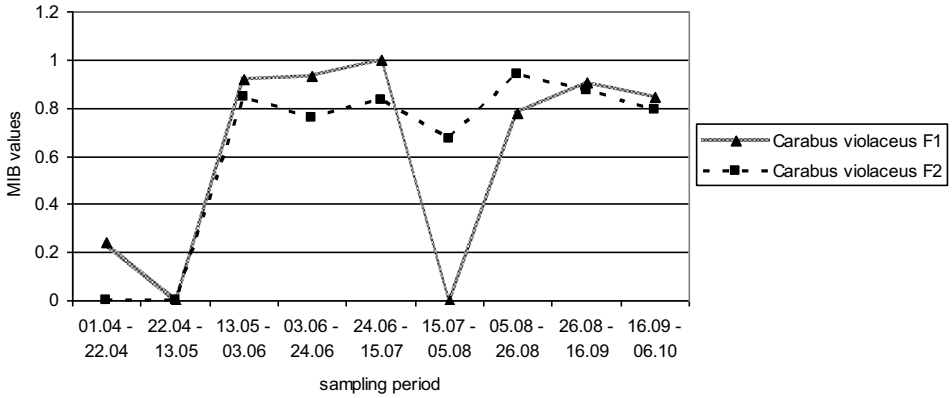


Fig. 9. The dynamics of *Carabus violaceus* MIB during 2007

Dynamics of the Shannon –Wiener index of diversity

The diversity of the carabid populations was evaluated using Shannon-Wiener index of diversity. Although the Shannon-Wiener index of diversity has indicated a higher diversity value in F1, the difference was statistically insignificant ($t=0.924$, $\alpha=0.05$). The higher species diversity observed in the case of the carabid populations identified in F1 site, could be explained by the higher number of species and high numerical abundance registered for the carabids from F1, compared to the ones from F2.

Table 3

The Shannon-Wiener index of diversity in the studied sites from the beech forest

| Studied site | No. of species | Shannon-Wiener index of diversity (H') | t ($\alpha=0.05$) |
|---|---|---|---|
| F1 | 25 | 2.181 | 0.924 |
| F2 | 14 | 1.855 | |

The diversity index values varied a lot during sampling period in each studied site (Fig. 10). In F1 forest I have noticed high species diversity values in spring and autumn. During summer (July-August) I have noticed a decrease of the diversity index for the carabid populations captured in F1 beech forest, due to the climate modifications from this period (the high temperature and the low humidity which are limiting factors for the ground beetles development), but also due to the estival diapause. In August-September period there was a slight increase of the diversity index values, followed by a decrease at the end of the study period.

In the case of the carabid fauna from F2 beech forest, I have noticed a maximum value for the diversity index registered in April-May. The diversity registered a decrease in June, followed by a slight increase during summer (July-August). This pattern of variation has been noticed before in the beech forest (Huidu 2011). That suggests that the values registered for temperature and humidity influenced positively the carabids development (Fig. 10).

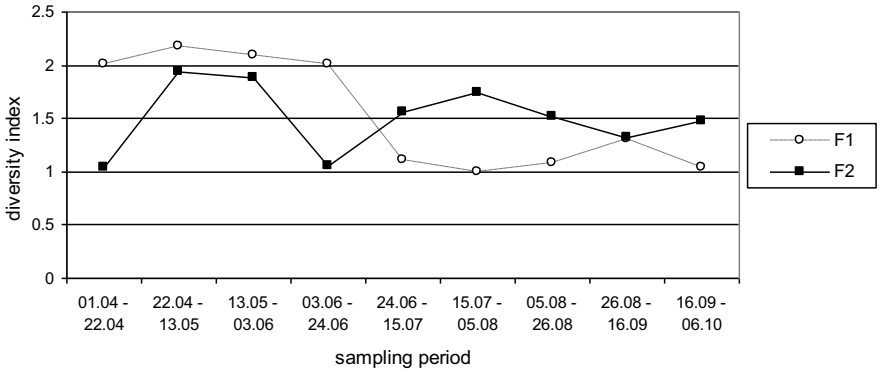


Fig. 10. The dynamics of the Shannon-Wiener index of diversity during the sampling period, in the beech forest (F1 and F2)

## CONCLUSION

In the two studied sites located in Bistriţa Gorges there were identified 28 carabid species (25 species in F1 and 14 species in F2), 11 of them being common.

Both studied carabid populations were characterized by the presence of a small number of (eu)dominant species (3 eudominant carabid species - *Abax parallelepipedus, Carabus arvensis, Carabus coriaceus*, 2 dominant - *Carabus violaceus, Carabus granulatus* in F1 site and 3 eudominant carabid species - *Abax parallelepipedus, Carabus violaceus, Carabus coriaceus* and 3 dominant - *Abax parallelus, Molops piceus* in F2), and a high proportion of accidental ones.

The sex structure of bought carabid populations was similar: a larger number of females captured mainly during spring.

A high numerical abundance was observed during spring and early summer in F1 and F2 suggesting that the majority of the identified species were spring breeders.

In the case of the carabid populations identified in F2 site it was observed a positive correlation between the abiotic factors (temperature and humidity) and species numerical abundance, the carabids development being favored by the registered values for temperature and humidity during studied period.

The dynamics of the species biomass for both studied locations was similar to the one of the numerical abundance, with a maximum point during spring. The (eu)dominat species *Carabus coriaceus, Carabus violaceus, Abax parallelepipedus, Carabus arvensis* had the highest contribution to the MIB total value.

The species diversity of the carabid populations from the studied sites was lower in the case of the ground beetle populations identified in F2 site, but the differences were statistically insignificant.

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# GENETIC IMPROVEMENT OF GROUNDNUT MORPHOLOGICAL CHARACERS THROUGH INDUCED MUTATIONS

Iancu Paula[1]

***Key words****: improvement, mutagenesis, groundnut, X rays*

## ABSTRACT

*Improvement in groundnut yield is normally attained through exploitation of the genetically diverse genotypes in breeding programs. Mutations, spontaneous or induced, are an important source for inducing genetic variability. Efficient mutant production systems, through physical mutagenesis, have been used rarely in groundnut in our country.*

*A vast amount of genetic variability for quantitative characters has been generated through experimental mutagenesis in the past 50 years all over the world. Mutant Variety Database (MVD) includes an important number of groundnut varieties with genetic variability generated through induced mutations. Mutants for morphological traits, high harvest index and high yield have been identified and characterized. One groundnut variety, Tamburesti, has been released for cultivation using induced mutations in groundnut in our country.*

## INTRODUCTION

In Romania, groundnut should be one of the major oilseeds crop and it should revolutionize rural economy and lift the socio-economic status of individual farmers or agricultural societies, at least in the South part of the country. It is mainly grown as oilseed crop and it occupies an area of about 2000 hectares with a production of 1200-3000 Kg/ha. As a result of high protein and fat groundnut has a multifold use and can be utilized domestically for meeting the acute protein deficiency in our country. The average yield of groundnut in Romania is 1.5-2 t/ha compared to world average of 2.2 t/ha. Narrow genetic base of cultivated varieties of groundnut and the lack of irrigation are the reasons for low productivity.

Groundnut (*Arachis hypogaea L.)* is cultivated in Romania only in Research Centres of Universities and occupies nearly 2000 ha (Nedelcu,N., 1999) and it is mainly cultivated in South Oltenia on the sandy soils from this part of the country. As comparison, in India, where groundnut is an important oilseed crop, used for food and feed, it is grown in an area of 6.45 million hectares with a total production of 6.57 million tons. This contributes to 26.6% of world's groundnut area and 18.5% of world's groundnut

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production. Groundnut occupies nearly 28.3% of the cultivated area and contributes 31.7% of the production of the total oilseeds in this country.

It is widely used as principal source of cooking oil, digestible protein, minerals and vitamins in many countries. About 80% of India's groundnut production is crushed for oil, 12% for using as seed, 5% for food and 2% for export. Seeds are consumed directly either raw or roasted, chopped in confectioneries or ground into butter (Anand M. Badigannavar, 2007).

Groundnut is an important grain legume in the world in terms of production and international trade. It is an economically important leguminous crop for oil, feed, and groundnut food products and has occupied a coveted place among the oilseed crops being cultivated all over the world. It is also ranked number two in world oil production and is widely cultivated in the United States, Brazil, Argentina, China and India, after soy bean.

## MATERIAL AND METHODS

This paper tries to present a short bibliographic presentation of the world realizations in groundnut mutagenesis, comparative with Romanian works in this field.

This article wants to present mutation breeding as a good variant of plants breeding, especially to groundnut, as concern morphological characters.

## RESULTS AND DISCUSSION

The history of groundnut can be traced back to South America. It is considered to be native from Argentina or Bolivia and has spread in many developing and developed countries of the world. Groundnut was introduced in Europe in XVI century and in Romania, Toropu, I. 1936 indicates the first tries of groundnut cultivation in Experimental Research Station from Valul lui Traian and starting with 1955-1956 it were grown on the sands and sandy soils from left of Jiu river by a small group of university teachers from University of Craiova, leaded by distinguished teacher Pop Liviu (Mitrea Ion, 1993).

The average yield of groundnut in Romania varies from 1.5-2 t/ha compared to world average of 2.2 t/ ha. The major constraints for low productivity of groundnut are narrow genetic base of cultivated varieties and the lack of irrigation. Along these, there also is non-availability of early maturing varieties, photoperiod insensitive high yielding cultivars carrying resistance to biotic and abiotic stresses. Narrow genetic base of cultivated varieties of groundnut was our concern and creating a degree of genetic variability available for selection using mutagenesis, can play an important role in overcoming yield barriers. Improvement in yield is normally attained through exploitation of the genetically diverse genotypes in breeding programs.

Mutations are an important source for inducing genetic variability. Improvement in single or few economic traits characters can be achieved with the help of induced mutations within the shortest possible time. Induced mutations have been widely accepted as a supplementary approach in the crop improvement program, thus speeding up the breeding programme considerably. Mutation breeding has played a significant role in the development of many crop varieties. In Romania, one groundnut variety (Tamburesti) has been developed using induced mutations (physical mutagenesis, gamma rays).

Some of the major research centers made important contribution in the field of induced mutation and development and release of mutant varieties in India are IARI (New Delhi), BARC (Mumbai), ICRISAT (Hyderabad), TNAU (Coimbatore) and NBRI (Lucknow). Recently Kharkwal and Shu, (2009), have reported that more than 343 mutant cultivars belonging to 57 plant species were approved and released in India.

A number of physical mutagens are widely employed to induce genetic variability in plants. Gamma and X rays (GR) are the most widely used physical mutagens in crop improvement. They are electromagnetic radiation (ionizing radiation) with the highest energy level. They are well known for their action in causing extensive damage to DNA.

Yield contributing traits such as: plant height, number of primary branches per plant, number of pods per plant, pod length, number of seeds per pod, days to flowering, days to maturity, 1000 thousand seed weight and seed yield per plant, are the metric traits which are quantitatively inherited and directly or indirectly contribute to increase in yield of crop plants. Their inheritance is controlled by multiple (poly) genes.

Mutation breeding in crop plants has its significance in selection of desirable genotypes and directly using them either as cultivars or as parents in recombination breeding programme. Efficient mutant production systems, through either physical or chemical mutagenesis, have been well established in groundnut. A vast amount of genetic variability, of quantitative traits, has been generated through experimental mutagenesis in the past 50 years.

Groundnut is one of the principal economic crops of the world, which has been exposed extensively to mutagenic treatments for induction of variability. The crop is suited for mutagenesis for several reasons: it is practically a self-pollinated species: the embryos are easily exposed to mutagens and have 6-8 primordia with buds in their axils making several potential targets available (Ashri, 1976a). Physical mutagens such as, X-rays (Patil, 1966), gamma rays (Lodha *et al*., 1985), beta rays (Jiang and Zhou, 1987) and fast neutrons (Shivaraj *et al*., 1962) have been used to create genetic variability in groundnut. However, gamma rays are the most widely used and most effective mutagens in groundnut.

Mutants have been obtained in groundnut either spontaneously or induced by physical mutagens for characters like, lethals, chlorophyll deficiencies, plant stature, growth habit, branching pattern, leaf, flower, sterility, stem, pod, kernel, pod yield, shelling percentage, days to maturity, seed dormancy, seed composition, oil content and quality, resistance to diseases and physiological characters besides cytological abnormalities.

Variation for morphological characters was found to be more predominant than other characters except pod (Patil, 1966; Reddy et al., 1987; Patil and Mouli, 1979a). Mutants with increased pod size were reported in different varieties (Ramanathan, 1979). On gamma irradiation of small seeded, high yielding and disease resistant variety Georgia Brown several large-seeded lines with high variability for disease incidence, pod yield, total sound matured kernels, pod weight, seed weight and seed size distribution were isolated (Branch, 2002). Early maturing types have been obtained on mutagenesis and in crosses involving mutants (Mouli and Kale, 1982).

Various physical mutagens had been tested for groundnut mutagenesis. The commonly studied parameters for the mutagen of interest include its dosage effect, optimum doses, mutation frequency and spectrum. The decision of using a particularly mutagen is not always based on its effectiveness, but on their availability, the convenience for treatment and post-treatment management. Studies on induced mutation in groundnut were first carried out by X rays by Gregory (1959). Few physical mutagens had been tested for groundnut mutagenesis during the past 50 years. The recommended optimum doses could vary significantly from one study to another since mutagenic effect can be influenced by genetic susceptibility and its physiologic status. The successful dose for groundnut varies from 100-450Gy, both for X and gamma rays. Several mutagens have been tried and proven to be capable of inducing mutations in groundnut. Gamma rays have so far been the most widely used mutagen; more than 80% of the mutant varieties were developed from mutants induced through gamma irradiation (http://www-mvd.iaea.org).

In Romania, researches applying ionizing radiations to groundnuts were started in 1974 using works of individual selection from a diverse source of germplasm (seed) consisting from Tamburesti population and an assortment of mutagenic import varieties such as Jelud, Velican, Braziliene. These were treated with different doses of gamma radiations. In this way it was realized to Tamburesti Research Station of University from Craiova, an important number of lines which were introduced in the state network for tries and variety omologation. These were: T 227, T 242, T 232, T 54 and T 55 (Marghitu Valeria, Chichea, I., 1982).. Then, the line Tamburesti 227 was omologated in february 1983 as variety under the name of Tamburesti. This variety presents tall plants, big number of nodosities, relativelly high number of branches, high yield and good quality. It was also obtained a few lines and among these T 55 emphasizes through yield increase and quality. As concern the influence of gamma rays upon the groundnut yield on hectare, these increase the yield, but insignificantly until 6000R and from 10000R up, the doses decreased substantially to Jelud, Velican and Braziliene varieties taken into study. The objectives of the studies were: obtaining some groundnut with short vegetation period, productive, of quality and resistant to diseases and less favorable natural conditions.

Experiments using doses of 6000 and 9000R of X and gamma rays to few varieties of native and foreign groundnut were conducted by Iancu Paula in the period of 2001-2005with the view to obtain PhD thesis. As a result of the influence of these doses in the three years it was determined that the experienced varieties can produce high variability as concern morphological characters which conduct to increased groundnut productivity. Mutagenic treatments have induced a great number of groundnut mutants with altered morphological characters, pod and seed characters.Mutagenesis represents an efficient method for groundnut improvement and the perspective lines obtained as a result of irradiation which outruns the control will be tested in the future years (Iancu Paula, 2005).

Groundnut mutants have been obtained all over the world, by physical mutagens for characters like, branching pattern, sterility, stem, kernel, pod yield, shelling percentage, days to maturity, seed dormancy, seed composition, oil content and quality, resistance to diseases and physiological characters. Variation for size, texture, type and modifications of leaf parts were found to be more predominant than other characters except pod (Patil, 1966; Reddy *et al*., 1987; Patil and Mouli, 1979). Pod variation ranged from a minute pod equivalent to as small a size as sorghum seed, to extra long pod having seed weight of 1.30 g (Patil and Mouli, 1979), which also included promising varieties. Mutants with increased pod size were reported in different varieties (Reddy *et al*., 1987; Ramanathan, 1979). On gamma irradiation of small seeded, high yielding and disease resistant variety Georgia Brown several large-seeded lines with high variability for disease incidence, pod yield, total sound matured kernels, pod weight, seed weight and seed size distribution were isolated (Branch, 2002). Early maturing types have been obtained on mutagenesis and in crosses involving mutants (Mouli and Kale, 1982).

Many of the mutants with extreme changes in morphological features like, mottled leaf mutant (Srivastava, 1970), imparipinnate leaf mutant (Mouli et all., 1989) recorded reduced pod yield levels compared to control. Although, mutation breeding programmes employed exclusively for disease/ pest resistance have been rather scarce in groundnut, a few mutants obtained in the projects proved to be resistant/tolerant (Qiu, 1982; Marghitu *et al*., 1982). The use of induced mutations in breeding for quantitative characters was also demonstrated by utilizing the induced mutants in hybridization (Patil, 1977; Balaiah and Reddy, 1977; Balaiah *et al*., 1977). Emery *et al*. (1965) stated that, even though induced macro-mutations were generally deleterious, the diversified genetic background created by irradiation could be a valuable source of germplasm that could be stabilized in normal

appearing phenotypes. Several mutants were utilized in hybridization programmes and many high yielding lines have been obtained as cross derivatives of the mutants (Mouli and Kale, 1982; Jiang and Zhou, 1987). Induced genetic variability of quantitative traits like maturity, plant height and yield components (pods per plant, seed per pod), were observed in many experiments. A number of mutants were generated with altered performance of quantitative traits around the world. However, most mutant varieties released for commercial production involved improved performance of quantitative traits like maturity, plant height, pods per plant, seed per pod and high yield.

According the FAO/IAEA Mutant Variety Database (http://www-mvd.iaea.org) from the total number of registered mutant varieties, a number of 71 varieties are only for *Arachis hypogaea* specie. From these, by physical mutagenesis it were obtained 65 varieties: 10 varieties (X rays), 41 (gamma rays), 12 (beta rays) and 2 varieties with laser influence. By chemical mutagenesis it were obtained 4 varieties (EMS) and one variety is represented by a natural mutant and one become from the crossing between one normal variety and a mutant one. Among those varieties, more than 80% were developed using gamma rays treatment. Most of the mutant varieties were selected for high yielding, high harvest index, early maturity, quality. Main countries which focus on groundnut mutagenesis are: China (30); India (28); Bangladesh (4); Malaezia, Argentina (2); SUA, Myanmar, Vietnam, Sri Lanka, Pakistan (1) (http://www-mvd.iaea.org).

Induced mutagenesis using physical mutagens has played a vital role for genetic enhancement of cultivated groundnut. More than 200 Trombay groundnut (TG) mutants affecting plant height, leaf type, plant type, pod type, seed size were induced by mutagenesis and by recombining favorable alleles from these mutants, 83 breeding lines having superior agronomic traits have been developed. Of these, twelve breeding lines including three direct mutants have been released for commercial cultivation across India country. These cultivars possess the desired traits like semi-dwarf habit, small dark green leaf, early maturity, high harvest index, high water use efficiency, fresh seed dormancy, large seeds, disease resistance, drought tolerance and wider adaptability (ICRISAT, 2008).

## CONCLUSIONS

The present investigation aimed morphological characters improvement in groundnut (*Arachis hypogaea L.*) through induced mutagenesis undertaken in the last 50 years by the Departments of Genetics and Plant Breeding of University of Agricultural Sciences from the countries which focus on groundnuts crop.

Mutation breeding programmes to induce variability to groundnut emphasized a large spectrum of modification in various doses of physical mutagen agents.

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# THE INFLUENCE OF X RAYS UPON CHEMICAL COMPOSITION OF GROUNDNUT SEED

Paula Iancu[1], Soare Marin[2]

***Key words:*** *X rays, chemical composition, seed, irradiation*

## ABSTRACT

*Groundnut is a well known plant for high content of protein and fat and also for superior quality of protein comparative with other oilseed crops. Because of that the attention of many researchers focus on breeding groundnut for obtaining varieties with seed rich in protein and fat. In the latest time, genetic researches and breeding used irradiations with X rays with the aim to obtain mutants conditioned by simple characters.*

*Irradiations of the seed with X rays in doses between 5000 and 10000 r had as result the increase of genetic variability of the quantitative characters, affecting in the same time qualitative characters. Depending also by climatic conditions, it will indicate that the increase of the irradiation effect is determined by these conditions and variety.*

## INTRODUCTION

Groundnuts are taking part from oilseed plants which present high quantities of fats and proteins that can be easily extracted in industrial way. There accumulation it can also produce in different parts of the plant and because of that it has forage value. Seed and plants are important sources of energy, both for human consumption and feed for animals, but also as raw materials for a wide range of industrial products. The value and usefulness of this crop is given in the first row of quality of the seed. In general, the beans peanuts contain 40 - 55 % fat and 20 - 30 % protein.

Groundnut is the world's fourth most important source of edible oil and third most important source of vegetable protein, groundnut and groundnut butter are whole foods that pack a lot of nutrition into just one serving. Groundnut contains high quality protein, which is important for children and people eating more meatless meals.

In the scenario of growing populations, which also needs balanced food and to solve deficiencies of protein, oilseeds are used to manufacture and market high protein foods at reasonably low prices (Bookwaltes et al., 1979).

Inclusion in the program for improvement of the plants of artificial induction of mutations, as a method of creating new variations in breeding has led to achieving outstanding results, which have resulted in the manufacture of numerous varieties of culture plants. Regarding the mutational experiences to groundnut over the years, it has been

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obtained a serie of mutants with valuable qualitative and quantitative characters. Inducing a broad spectrum of genetic variability by using physical and chemical mutagens factors can help improve crop.

## MATERIALS AND METHODS

As biological material we used varieties like Tamburesti (small-sized) and Venus (large-sized). Dry seeds were treated with roentgen rays in doses from 5000 r - 10,000 r in S.C Electroputere S.A. Craiova in 2010-2011 years. These doses were considered effective if the forementioned varieties, based on previous experiences (Iancu Paula, 2008).

In this paper will present the effects of radiation on seed quality of groundnuts from plants obtained from seeds irradiated and grown on a sandy soil in SD Tamburesti. As control it were used seeds of the same varieties, but not irradiated.

Character study of this experience was based on laboratory measurements. Percent of dry substance, protein and fat of the samples was performed by the Laboratory of Chemistry-Physiology of IBNA Balotesti using a method permissible Official Journal of the European Union 26.02.2009 EN L45/1 and AOAC Official Methods of Analysis (2005) and an HPLC apparatus.

## RESULTS AND DISSCUTIONS

The Laboratory of Chemistry-Physiology of IBNA Balotesti determined first the content of dry substance at 103°C (%), the brute protein and fat content (% DS) and then profile of amino acids (g/100g DS) (fig. 1,2,3,4).

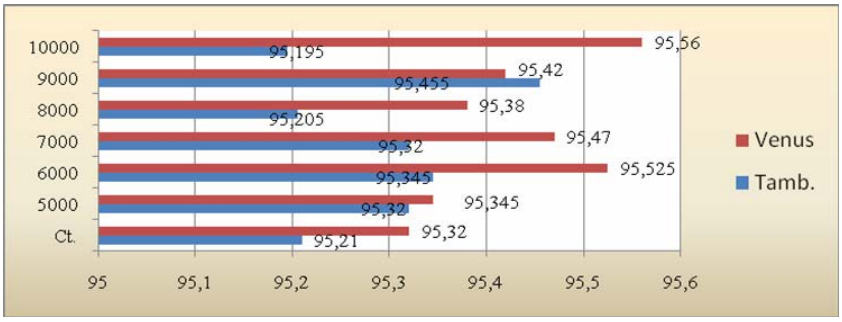


Fig. 1. Quantity of dry substance from experimented variants

In 8000-germplasm accessions analyzed at ICRISAT, a range of 16 to 34 percent protein was observed. However, these ranges of variation were not maintained when selected genotypes with such variation were tested over season and locations (Dwivedi et al., 1993). Pancholy et al. (1978) reported crude protein content of whole seed groundnuts ranges between 22 and 30 per cent showing a large variation, which is greatly influenced by genotype and environments. Sugui (2004) has released a new groundnut variety NSIC Pn-12 in Philippines. It is a selection from an advanced breeding line ICGS (E) 27, acquired in 1986 from ICRISAT, Patancheru, India. It contains 26.26 per cent crude protein. Liao et al. (2004) reported the release of black seed coat groundnut cultivar Zhonghua 9 which contains 28.3 per cent for chemical traits such as low oil, high protein and sugar. The genotypes DCG-24 (TG49 × R-9227, 8), DCH-26 (TG49 × R 9227, 19) and TKG 19-A were reported to be superior (Yashoda, 2005). Yugandhar (2005) evaluated 196 accessions for protein content and selected fourteen accessions for high soluble protein.

In our experiment, the effect of radiation on protein content is more obvious in irradiated variants. Influence of X-ray (fig. 2) is manifested by an increased accumulation of the protein at low doses of radiation. Crude protein content is highest in the variant of irradiation with 6000 r, reaching in the two years of experimentation at an average of 30.47% for Tamburesti variety, exceeding control to 1.97%. Also, this variety, in the irradiation dose increases, the protein content decreases, reaching the dose of 8000 r from an average lower by 1.12% than control. We find that the maximum dose of radiation (10000 r) has not negatively affected the protein content in this version recorded an average of 28.71 g higher than that of version control with 0.21%. Analyzing the crude protein content to Venus variety, it was found a positive effect in the way of increasing the amount of protein up to 27.02% only in the variant irradiated with 6000 r.

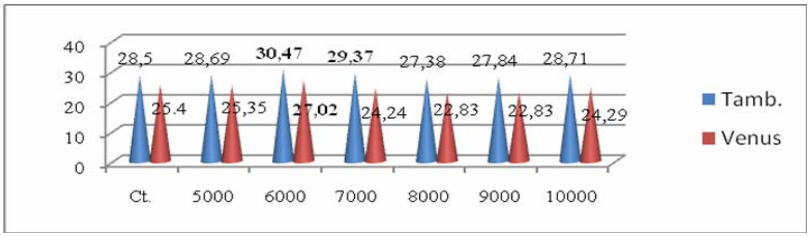


Fig. 2. Crude protein content of the irradiated varieties

In the other variants of irradiation, protein amount decreases comparative to control value of 25.4% until 22.83% (8000 and 9000 r) which means 2.57% lower. The amount of protein decreases in the favor of fat content (fig. 3) which in 9000 r variant has a value of 44.52%, up to 4.22% higher than the control. Protein content has a significant inverse correlation with oil (Dwivedi et al., 1990).

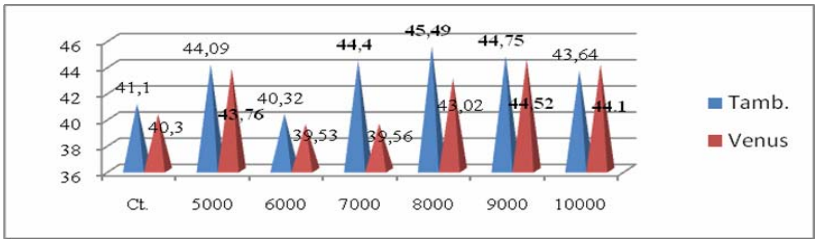


Fig. 3. Crude fat content of the irradiated varieties

It was also determined the essential amino acids content (fig. 4). Total essential amino acids in the experimented variants presents higher amounts comparative with control variants in both varieties. Tamburesti control variant presented 4.53g/100g DS and was outrun of all irradiated variants. In the irradiated variants, the values of essential amino acids varied from 5.151g/100g DS (9000 r variant) to 6.104g/100g DS (10000r variant). For this variety smaller (5000r), middle doses (7000r) and even maximum dose (10000r) of irradiation presented positive influence upon the increase of amino acids content.

Venus variety reacted differently and not very favorable. Control variant presented a value of 4.68g/100gDS and was outrun by the variants irradiated with 5000, 6000, 9000 and 10000r. The irradiated variants with 7000 and 8000 r presented the lowest values, 4.61g/100gDS, respectively 4.60g/100gDS.

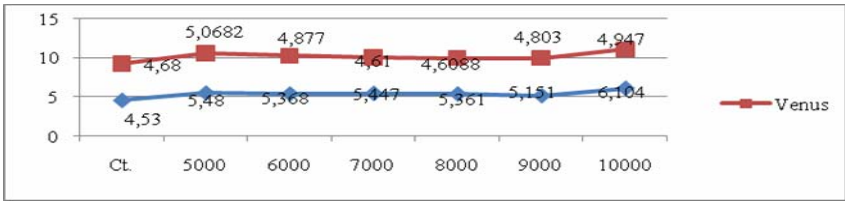

Fig. 4. The content of total essential amino acids

These values are substantially better comparative with the data obtained in a similar experience (Iancu Paula and co., 2008).

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✔ *Ingineria mediului*

**Vol. XVII ( LIII ) - 2012**

# STUDY ABOUT SEED-SET FLOWER HEAD VARIATION BETWEEN POPULATIONS OF CANADIAN THISTLE- *Cirsium arvense*

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***Key words***: *Cirsium arvense weed, seed-set, female clones, male clones, variation.*

**ABSTRACT**

*Although the efficiency of the sexed reproduction of the species Cirsium arvense is situated at well-balanced to low levels although her spread and gaining in light of new habitats, is constantly growing. It is possible that the seed-set flower head (S-S FH), able to spread, may succeed in doing this if it has got a large range of information, as a result of an outcrossing and by the seedling' stamina which derives from morphologically well-selected seeds, that is bigger, fuller and heavier. In the present study, morphological variability of the S-S FH from the female capitula is being analyzed according to their prolific location within the big inflorescence. Thus, flower head (FH) from the 1st to the 3rd branch have been analyzed, then those from the 4th to 6th branch and the 3rd category from the 7th to 11th branch. The big and heavy seeds have been formed on the first ramifications. Between the no. of formed seed and their total weight, a negative correlation has been found. In this correlation one might find an explanation in favoring natural and genetic formation of less but heavier seeds as a guarantee for the seedling's success in various habitats.*

## INTRODUCTION

Canadian thistle- *Cirsium arvense L./Scop.* is a widespread species in the Temperate Zone. Being a perennial plant, it insures its reproduction through vegetative propagation and sexual one. Its spreading takes place on the micro-level through its under-ground roots and on the macro-level through its seeds of type achene (Heimann & Cussans, 1996). The seeds are being formed within a complex sexual system (Table 1). The system is of dioecious mating type (Lloyd & Myall, 1976). This requires the existence of female and male plants. The respective plants form offshoot stems named clones. Female clones are pollinated compulsory by the male ones (Sagar & Rawson, 1967; Moore, 1975; Kay, 1985). This pollen carries out important genetic information and varied. Seed-sets from FH of female clones are variable and generally speaking reduced because of multiple causes. Among the most important factors which adjust the seed-sets one can mention: genetic factor and pollination agents' mobility= insects. Important factors are: sexual differences they can be enclosed or remoted, the seed-predators and the degree of filling up with endosperm. The less important factor is sexual rate, which can be equal, of 3 to 1 or more

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(Van Leeuwen, 1987). The male clones are genetically varied (Lloyd & Myall, 1976). The male clones also form seed-sets and according to their number per set they can be defined as : hermaphrodites with sets of over 10 seeds a FH, subhermaphrodites with reduced sets, 1-10 per FH and clone only with pollen, that is without seeds. Among various types of clones, there are also large hybridizytions outcrossing. As a result of the female flower fecundation, seed-sets should contain genetic information as varied as possible. In the present study the variability has been obtained from the diversity of populations, diversity of sex-differences, diversity of FH depending on their location on floriferous branches.

Table 1

Sexual cycle structure of *Cirsium arvense* weed

<table>
<tr><td colspan="6">MATING DIOECIOUS SYSTEM</td></tr>
<tr><td rowspan="4">FEMALE PART</td><td>Female plants</td><td>Pollination</td><td colspan="2">Male plants</td><td rowspan="4">MALE PART</td></tr>
<tr><td>Morphologically hermaphrodites</td><td></td><td colspan="2">Morphologically hermaphrodites</td></tr>
<tr><td>Female clones</td><td>Pollen</td><td colspan="2">Male clones</td></tr>
<tr><td>Little seed-set number</td><td></td><td colspan="2">Little seed-set number</td></tr>
<tr><td colspan="3">Female ecological conditions:</td><td colspan="2">Male possible way: initial gynodioecious population</td></tr>
<tr><td>a.Very important</td><td>b.Important</td><td>c.Less important</td><td rowspan="2">a-male clones with pollen no seed, b-subhermaphrodites with pollen and 1-10 seed-sets</td><td rowspan="2">c-hermaphrodites with pollen, over 10 seed-sets</td></tr>
<tr><td>i)genetic factor, ii)pollinator mobility</td><td>i)distance & sexes, ii)seed predators, iii)% endosperm,</td><td>i)sexual rate</td></tr>
<tr><td colspan="6">Large outcrossing</td></tr>
<tr><td colspan="3">Genetic variability</td><td>&</td><td colspan="2">Reduced seed-set number</td></tr>
<tr><td colspan="3">Genetic diversity</td><td>&</td><td colspan="2">Adapted plants</td></tr>
<tr><td colspan="6">NEW HABITATS</td></tr>
</table>

## MATERIAL AND METHODS

In order to determine the seed-production of *Cirsium arvense* a few populations from extended specific spreading area from the South of Romania has been chosen.

Selecting ecotypes: every population has registered a good growth on a large area, measuring over 100 sq.m. each, with high densities of stems/clones. Both female and male plant populations have developed in the fields tilled. On the female clones a number of 7 ecotypes has been selected (AF1-AF7) differentiated in 3 categories depending on the distance between pollination agents. Thus, 3 of the ecotypes had got pollination agents on an average distance under 50 m, another 3 with related pollination agents, at population' border and an ecotype with male clones at a large distance, 150 m. Among female ecotypes, different colors have been noticed (table 2). Male ecotypes summing up 3 (AM1-AM3) were also distinctive. The 1st ecotype has been chosen from the first 3 with an average distance under 50 m from the female plants; the 2nd has been placed near female clones and 3rd at a vary large distance from female receivers.

Taking samples: they went in a zigzag direction that crossed the respective population, without trying a stationing-positioning right in front of a certain plant. A stationing has been done at every 2 steps and FH have been harvested out of the clone.

Every referred clone was planned to be well grown and developed with big inflorescence. The FH have been harvested separately from every 3 different levels, out of the first 1-3 floriferous branches, then from 4-6th branch and the third category out of 7-11th branch. This procedure has been 50 times reiterated, separately on the 3 types of floriferous branches.

Table 2

The *Cirsium arvense* ecotypes diversity

| No. | Ecotype | Flower heads color: % purple (P)/ % green (G) | Distance from male / female flowers, m | Habitat |
|---|---|---|---|---|
| A. Female clones | | | | |
| 1. | AF 1 | 5 P/95 G | 25 | winter wheat |
| 2. | AF 2 | 15 P/85 G | 30 | sunflower |
| 3. | AF 3 | 65 P/35 G | 45 | maize |
| 4. | AF 4 | 20 P/80 G | 0 | winter wheat |
| 5. | AF 5 | 0 P/100 G | 0 | clover |
| 6. | AF 6 | 0 P/100 G | 0 | winter wheat |
| 7. | AF 7 | 0 P/100 G | 105 | winter wheat |
| B. Male clones | | | | |
| 8. | AM 1 | 100 P/0 G | 35 | winter wheat |
| 9. | AM 2 | 100 P/0 G | 0 (at the edge) | winter wheat |
| 10. | AM 3 | 100 P/0 G | 105 | winter wheat |

Analysis of seed-sets**:** the FH, one by one, according to ramifications' category and clone, that is, 50 per cent population multiplied 3 ramifications multiplied 10 populations equal 1500 FH have been harvested after stems in proportion of about 95 per cent within 12 days after its full flourishing on July. They consider that achenes have already been formed and developed accordingly (Derscheid & Schultz, 1960). The samples have been put to dry in lab conditions for 4-5 days. The FH had been weighted according to categories of floriferous ramifications, seed-sets from every FH have been counted separately and also weighted. The sterile FH had been recorded separately. The estimation has been made with the help of ordinary methods.

## RESULTS AND DISCUSSIONS

Variability of seed-sets and of sterility**.**The analysis of *Cirsium arvense*' population emphasized a specific variability. In the case of female clones, achene-sets varied both as interval (min to max) but also as location in inflorescence. On the harvested capitula from 1st-3rd branch, seed-sets were between 1-54(AF4) and 1-85(AF2). On the 4th to 6th branch, sees-sets varied between 1-56(AF1) and 1-78(AF4), and on the 7-11th branch between 1-35(AF3) and 2-87(AF5) (Table 3). On the male clones, the AM1 ecotype had no seed-set; AM2 ecotype formed 6 achenes in only one FH on the 1st to 3rd branch and 1-7 achenes in 2 FH on the 4-6th branch. The 3rd ecotype, AM3 formed achenes, only in 2 capitula with 1-6 seeds on the 1st-3rd branch. The FH' sterility has been extremely different. On the female clones the 1st-3rd branch had 8-40 percent sterility; the 4-6th branch had 12-64 sterility, and 7-11th branch between 18-82 percent without seeds. On the male clones, the sterility was between 84-100 %, which shows the prevalence of the pollen type versus subhermaphrodites type (1-7 seed-sets). As for sexual differences, the seed-sets were between 1-84, 1-71 and 1-67 on a middling-average distance from the male clones : between 2-83, 1-78 and 2-87 on the enclosed clones and in between 1-62, 1-58 and 1-72 at a great distance from the pollination agent.

Table 3

The seed-set number limits and sterile flower heads in *Cirsium arvense.*

| No. | Ecotype | Seed-set number limits | | | Sterile flower heads, % | | |
|---|---|---|---|---|---|---|---|
| | | branch no. | | | branch no. | | |
| | | 1-3 | 4-6 | 7-11 | 1-3 | 4-6 | 7-11 |
| 1. | AF 1 | 2-72 | 1-56 | 1-67 | 34 | 38 | 66 |
| 2. | AF 2 | 1-84 | 1-71 | 2-61 | 40 | 64 | 70 |
| 3. | AF 3 | 1-66 | 1-63 | 1-35 | 26 | 52 | 82 |
| 4. | AF 4 | 1-54 | 1-78 | 1-82 | 12 | 24 | 56 |
| 5. | AF 5 | 2-83 | 1-73 | 2-87 | 8 | 12 | 18 |
| 6. | AF 6 | 3-70 | 1-74 | 3-60 | 8 | 14 | 46 |
| 7. | AF 7 | 1-62 | 1-58 | 1-72 | 34 | 30 | 44 |
| 8. | AM 1 | 0 | 0 | 0 | 100 | 100 | 100 |
| 9. | AM 2 | 6 | 1-7 | 0 | 88 | 84 | 100 |
| 10. | AM 3 | 1-6 | 0 | 0 | 86 | 100 | 100 |

At first sight one cannot clearly divide any influence of the capitulum's branch location and of the sexual differences over seed-production. It has been emphasized the growth of sterility percentage towards the base branches and through distance increase versus pollination agent.

Variability of capitula' morphology and seed-sets per FH**.** The female clones formed different fertile flower heads (FFH) according to their location on the floral branches (Table 4). The average degree of fertility has registered higher value on the 1st-3rd branch, particularly 77 %; the 4-6th ramifications had FFH of about 67 % and on the 7-11th of only 45 % fertility. The conclusion is that analyzed Canadian thistle populations, 3/4 of the capitula from the 1st-3rd branch form seed-sets; 2/3 of middle capitula are fertile but those from the base form only 1/2 of capitula, seeds. The FH' weight was considerably equal, as well the populations' variability and its shows a good ecotypes' constancy. As for absolute values the FH' weight was in between 0,21-0,22 gr. The average number of seeds shows values in between 26,3 on the 1st-3rd level; 24,8 seeds/FH on the 4-6th level and 25,5 seeds/FH on the 7-11th level. Achenes-set had an average total weight in between 0,0195 gr. on the first ramifications, 0,0150 gr. on the middle and 0,0149 gr. on the ground ramifications. The one thousand-grain weight (TGW) was in between 0,76 gr. from 1-3rd branch; 0,60 gr. on the 4-6th branch and 0,59 gr. on the 7-11th branch.

Correlation between various morphologic characters. According to the increase of the FFH' percentage seed-sets production has been positive (Fig.1). The estimation shows a growing rate of 2,34 seed at every 10 % fertility growth.

Table 4

The flower head and seed-set per head variability in *Cirsium arvense*.

| Branch position | FFH * | | FFH weight | | Seed-set number | | Seed-set weight | | TGW*** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | % | SEM ** | g. | SEM | no. | SEM | g. | SEM | g. | SEM |
| | Female clones | | | | | | | | | |
| 1-3 | 77 | ± 5.13 | 0.22 | ± 0.019 | 26.3 | ± 3.05 | 0.0195 | ± 0.1770 | 0.76 | ±0.055 |
| 4-6 | 67 | ± 7.28 | 0.22 | ± 0.018 | 24.8 | ± 3.40 | 0.0150 | ± 0.5671 | 0.60 | ± 0.038 |
| 7-11 | 45 | ± 7.91 | 0.21 | ±0.020 | 25.5 | ± 3.72 | 0.0149 | ± 0.5631 | 0.59 | ± 0.037 |

* FFH = fertile flower head ; ** SEM = standard error of mean;
***TGW = thousand grains weight

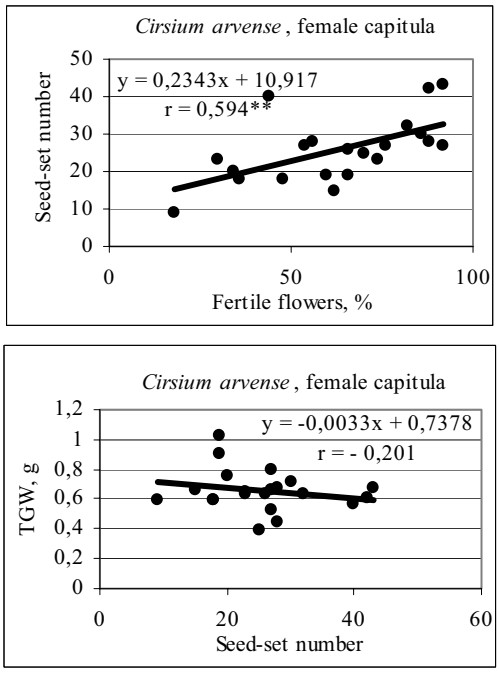


Figure 1. Correlations between *C. arvense* female fertile flowers and morphological seed-sets characteristics.

The correlation shows a favorable tendency of *Cirsium arvense* population to form more seeds by increasing the number of FFH. The formed seeds had absolute weight a little influenced by the degree of FH' fertility. The correlation between number of seeds and their total weight (TGW) was negative and statistically not insured. Out of the antagonism of the two morphologic characters it resulted a diminished value of TGW at every 10 achenes additionally formed. A smaller number of seeds bring about the increase of their absolute weight. The correlation might explain the inefficiency of sexed reproduction on *Cirsium arvense* through a smaller number of seeds in order to their being heavier.

## CONCLUSIONS

The recurrent analysis of populations within a specific spreading area for a species, brings in momentary information in the evolution's dynamics of the *Cirsium arvense* weed. The variability in literal sense, may refer to genetic dowry of the genets as well as to morphological one with the structure of the FH' seed-sets. FFH of the female clones formed the highest percentage in the upper part of the branches. The reference material shows a great diversity in estimating FFH (Detmers, 1927; Salisbury, 1942; Parsons, 1973) but in no way, has it displayed their variability according to their location on the floriferous branches. The seeds are being formed either into the female clones' flowers

or into the male ones. The sets in a reduced number prove a more advanced and evolutionary stage.

The analysis of seed-sets that the plant produces, brings in some momentary information over morphological diversity of populations within a specific zone. As part of female ecotypes, sterility has recorded 8-82 % and within FFH the seed-set was up to 1-81 pieces, with less obvious variations among the group of female plants.

Achene-set per FH and TGW show a very important negative connection that is the tendency of forming less numbered achene-sets but having a bigger TGW.

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# POLLUTION OF SOIL BECAUSE OF UNCONTROLLED ANTHROPOGENIC ACTIVITY

Marian Mădălina[1], Neblea Monica[2], Bonța Ioana Andreea[3]

***Key words*:** *ecopedological factors, pollution, residuum petroleum, salty water, degraded soil.*

## ABSTRACT

*The purpose of this study was to determine oil and salt water pollution of land and finding the improvement of the methodology. In the field phase were made surveying and were collected samples from soil and water. The physical and chemical determinations of the samples were made in the laboratory. Data interpretation was done according to Elaboration Methodology of the Studies Soil/ 1987, Order MMGA no. 161/ 2006, Order MAPPM no. 756/ 1997, MAAP Order no. 223/ 2002. The soil studies, that have evaluated ecopedological factors, soil cover and laden with pollutants of land, showed that on the studied plot appeared two types of pollution, after the exploitation and transportation of crude oil: chemical pollution (with oil and salt water- mixed pollution) and physical pollution (through drilling activities, mining and transportation of crude oil).*

## INTRODUCTION

This paper gives an assessment of joint polluted land by oil and salty water due to crude oil and petroleum processing and transport. Pollutants from these sources affect air, surface water and groundwater, vegetation and specially soil. It shows how the pollution is being made and its effect on the soil, the degree of pollution is being determined, solutions to remedy soil pollution by physical and chemical methods are being proposed. Global population growth will necessitate agricultural expansion within the next years (Tillman et al., 2002).

## MATHERIAL AND METHODS

The study was concluded in four phases. In documentation phase, data and information about environmental elements from specialized literature were collected, and materials that were previously developed by OSPA Arges, ICPA Bucharest and SC ALFRID SRL Pitesti have been studied. The next phase was land phase in which samples of soil and water was collected. In the same period, topographical measurements to outline the shape and all elements of detail, including erosion formations, have been made. During the measurements, a SOUTH NTS-350 total station was used, which is produced by South Surveying. Was also used SOKKIA GPS, Stratus model, receivers that have 12 channels, L1 signal, C/A code, 5m-1m precision, 5mm, internal antenna. In laboratory phase, physical and chemical measurements were made and in office phase, data from field and

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laboratory have been systematized and the results were interpreted. Based on this interpretation, improvement methodology has been developed.

For chemical characterization and identification of the level of pollution of surface water, groundwater and stagnant water, 4 samples were collected from Strîmbu stream, and from a number of existing swamps of land taken into research. Sample A1 is the source of the coastal slope, water in which A2 probe A3 stagnant surface water including VI and IX, A4 Strîmbu stream surface water north of the square IX. The research was conducted in two years, the samples being harvested by OSPA Arges and ICPA Bucharest. To the water samples, 6 soil samples (P1, S1, S2, S3 – in 2011, S4.1 and S4.2 –in 2010) were added. (figure 1). Pedological study has given information on the state of land quality and level of pollution, offering observations on changes produced in a year.

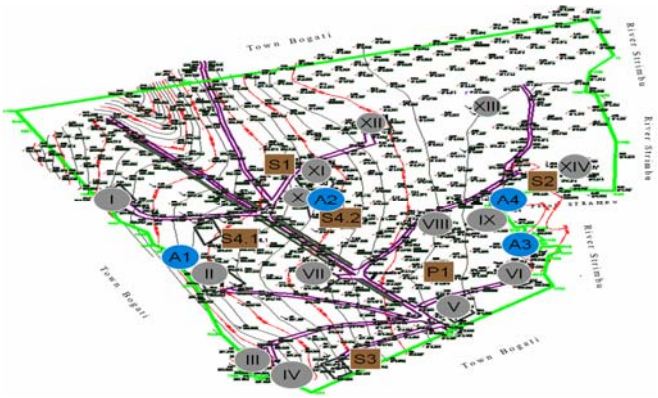


Figure 1. The location of existing wells in the field and soil and water samples.

In the water samples were determined: the total content of salts, anions and cations content and total hydrocarbon content from petroleum. The classification of chemical elements determined in water samples collected as quality indicators, was performed according to MMGA order no. 161/16.02.2006. Element considered for system acidification was pH and salinity were the mineral residue and Conductivity at 105°C, chlorides, sulphates, calcium, magnesium and sodium. To fit the contents of hydrocarbons in water samples using the STAS 4706/1974 stating that any groundwater or surface water that contains more than 0.1 mg/dm3 hydrocarbon oil is considered polluted. Classification in class was based on the ranking scale oil polluted water. (Toti et al., 1999). Harvested water salinity regime within them graded from I (very good), II (good), III (moderate), IV (poor) to V (bad) to different chemicals and mineral residue (according to order MMGA no. 161/2006).

In the soil samples were determined the following physical, hydro and chemical properties: granulometric fractions, apparent density, total porosity, standard penetration resistance, initial soil moisture, saturated hydraulic conductivity, organic matter/total organic carbon, pH site, total nitrogen, mobile phosphorus, exchangeable cations, total exchange acidity, cation exchange capacity total, total content of salts, the anions and cations content, available potassium (mobile) and total hydrocarbon content from petroleum. We also analyzed the legal situation of land, being 157039mp total area, of which: 133040mp pasture, 8933mp access roads, 15066mp petroleum sounder.

## RESULTS AND DISCUTIONS

According to the residual mineral content (mg/l) water source under the slope will fall in quality class (poor) due to the high content of salt (9882 mg/l). The circulation profile rainwater is fast, has different infiltration rates, transport of pollutants reaching greater depths while polluted water and can contaminate rivers flowing (stream Strimbu). The ground stagnations occur or where squares are the areas waterproofed probe due to oil pollution (table 1) or in areas with subsidence resulting from uncontrolled movements.

Table 1

Chemical characteristics of water samples collected OSPA (2010)

| Sam -ple | Elements analyzed | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $HCO_3^-$ | $Cl^-$ | $SO_4^{2-}$ | $Ca^{2+}$ | $Mg^{2+}$ | $Na^+$ | $K^+$ | Cond. el. | Res. cond. | Res. mineral |
| | mg | mg | mg | mg | mg | mg | mg | µS/cm | mg | mg |
| A1 | 311 | 6083 | 48 | 768 | 162 | 2484 | 27 | 17070 | 11608 | 9882 |
| A2 | 119 | 530 | 33 | 51 | 5 | 286 | 27 | 1850 | 1221 | 1051 |
| A3 | 143 | 82 | 10 | 19 | 1 | 79 | 14 | 438 | 289 | 348 |
| A4 | 195 | 61 | 10 | 56 | 8 | 34 | 5 | 453 | 299 | 369 |

Soil type is Luvisol hipostagnic identified, sometimes vertic presence of textural Bt horizon rich in clay migrated from the upper horizons, where he formed a luvic horizon. Bt horizon, vertic is sometimes a barrier to infiltration of rainwater and from accidents. Accumulates on the surface of a sheet of water polluted or not, moisten the upper horizons reaching the soil surface. Humus content of soil is somewhat uniform and depends on the one hand nature of the soil and the workload of its crude oil and on the other, to locate the point of harvest can be grassy field, in places free from natural or anthropogenic reasons or soils with crust made from petroleum fractions at various stages of degradation.

The textural analysis of profile P1 is clear textural differentiation at 39cm depth, between El horizon and horizon Btw. In the eluvial horizon and Btwy horizon to a depth of 110cm, clay content exceeds 50%. The depth of 39-75cm it reaches 57%, exceeding the limit textural differentiation index of 1.2. The depth of 75-110cm, although the clay content decreases towards the top of the Bt horizon, it remains above 50%. In surveys S1 and S2 pebble content is 6-7% up to 50cm where the percentage increases around 35-40%. At S3, the gravel horizon is found at 60cm depth. Soil material from the three surveys is of medium clay, containing varying amounts of sand. Profile P1 was harvested from an area that had a seam-clay loam and gravel is low, unlike the rest of the soil developed on a bed of gravel S1, S2, S3, or sandy lenses appeared -grounds. Apparent density is low on 0-5cm, medium-large at depths between 55-90cm ranging from 1.49 to 1.65 g/cm3. Total porosity is high on the 0-5cm (47.6% v/v) it fell to the middle and lower 55-60cm in the profile (37.8% v/v). Penetration resistance is low the first two sampling depths, values increasing from surface to depth (13 or 22 kgf/cm2). Saturated hydraulic conductivity after the first has values close about 0-60cm sampling depth increases to 85-90cm. All values fall within varying large class of 35.51 mm/h to 42.18 mm/h. The P1 profile has an average content in total nitrogen on 0-20cm (0.209%) low on 20-40cm (0.126%) and very low to 110cm (<0.1%). Surveys S1, S2 and S3 are content to 0.1% (very small) which decreases from surface to depth. At P1, S1 and S2, the contents Na did not change are very small, less than 1 me/100 g soil, meeting the highest value on the horizon of 50-70cm of P1 (0.61 me/100 g

soil). The texture of the soil is clayey at this depth, this seam can accumulate sodium because of the higher clay content below 0.002mm. The profile P1 is non alkaline, $V_{Na}$ values ranging between 0.7 and 4.3%. The highest value of 4.3% is on the depth of 90-110cm, values starting to grow down from 50cm. The S1 and S2 survey are also non alkaline, $V_{Na}$ values ranging between 0.6 and 2.6% being higher in the S1 profile. The S3 survey shows a higher $V_{Na}$ especially on the depths of 20-40cm and 40-60cm.

Table 2

Total petroleum hydrocarbon content in soil samples P1, S1, S2, S3. ICPA (2011)

| | Depth | Attempts required THP | |
|---|---|---|---|
| Sample | (cm) | mg/kg | % |
| P1 | 0-2 | 150 | 0.02 |
| | 2-18 | 450 | 0.05 |
| | 20-40 | 500 | 0.05 |
| | 50-70 | nd | nd |
| | 75-90 | nd | nd |
| | 90-110 | nd | nd |
| S1 | 0-20 | 250 | 0.03 |
| | 20-40 | nd | nd |
| | 40-50 | 700 | 0.07 |
| | 50-60 | nd | nd |
| S2 | 0-20 | 200 | 0.02 |
| | 20-40 | nd | nd |
| | 40-60 | 150 | 0.02 |
| S3 | 0-20 | 450 | 0.05 |
| | 20-40 | 100 | 0.01 |
| | 40-60 | nd | nd |
| | 60-80 | nd | nd |
| | 80-100 | nd | nd |

Soluble salts reach depths of over 100 cm salinity set aside for upper horizons. For periods of drought in the Bt horizon of luvisols crack, followed by a rainy period in which the cracks are filled with water to dissolve the salts in the soil and then rises to the surface evapotranspiration, soil improvement potential in superficial layers leads to no avail. On the other hand in rainy periods water can accumulate in the Bt horizon, can reach the surface. Thanks so much better drainage stagnating less excess water from the soil surface, especially in the ravines. In periods of heavy rain water accumulated in the squares of the probe is running out to the river, stagnating in microdepression or infiltrating into the soil. Strimbu Creek has two loops that penetrate inland drainage contributing to the plot. To flow up the river is considerable connects to ground water, salts contained in the stream water is taken. Was charged extreme wedge is partially flooded stream waters taking existing salts to the soil surface from stagnant water.

Profile P1 is non salinized according conductometric residue, content of soluble salts in the profile ranging from 25 to 6 mg/100 mg/100 g soil and ground to a depth of 75-90 cm. Content of sodium is reduced, but their variation profile that we give indications to the content of clay, coarse sand and dust infiltration water flow can stagnate or salts to contaminate soil and then leaving. The presence of salts, even in small quantities, is

indicative of pollution. Sodium content increased the depth of 90-110 (4.2 mg/100 g soil) in complete correlation with the residue of 18 mg/100 g soil Conductivity. S1 and S2 are assigned survey because results conductometric residue at non-salinized. The difference in sodium content between S1 and S2 can be explained by the location of these two surveys, a top secondary S1 flattened the Glacis and S2 in the flat meadow brook Strimbu. At high stream flows dilute sodium in S2. S3 survey, is saline poorly on 0-40 cm (118 respectively 132 mg/100 g soil mineral residue) and moderate to 40-60 cm (311 mg/100 g soil). Below this depth of soil material has more gravel lutoargilos leading to drainage far more intense that the amounts of salts, salinized become weak. Sodium content increases from 33 mg/100 g soil surface to 40-60 cm (107 mg/100 g soil). Below this depth decreases due to gravel (87 mg/100 g soil) on the 80-100 cm depth.

The main problem in this area is the surface and deep circulation of meteoric water, spring water from coastal and industrial water, water leaking into the soil besides polluting Strimbu brook that runs through this course and surface waters.

Infiltrated crude oil was degraded under the action of natural factors; of great importance is the microbiological activity of bacteria and fungi from the native micro-flora, which have degraded various fraction of crude oil. A part of asphaltenes contained in the crude oil that have entered maximum of 5-7 cm in the ground surface, or have remained at the surface forming a crust above the ground, can be seen also today on little surfaces, being generally fragmented, but consisting an evidence of pollution with this product. The fact that even in small quantities hydrocarbons were detected by analyzes conducted at depths of 100-110 cm is further proof that even at these depths, and perhaps more deeply, oil has penetrated in the time of early mining and in the case of inherent accidents.

At the poll S4.1, the level of pollution with oil residue is very small 0-20 cm (0.145%) and very small with values below 0.1% up to 60 cm. At the poll S4.2, the soil has a low content, up to 40 cm, with values over 0.160%.

Oil pollution, studied on a period of two years, is quite low taking into consideration that the originally pollution was produced long time ago, the discharged quantities of oil were initially reduced (initial installation of drilling wells) and during the time the pollution was caused by the discharge from the squares of probe, or by the cracks of the pipelines that transported crude oil to the parks. Over the time, some of the spilled oil has penetrated the soil, a part has flowed together with the surface and groundwater in the main collector (Strimbu river) and in some cases has been immediately removed after possible accidents by the maintenance personnel by emptying, partial stripping of topsoil and transportation of the polluted material, followed by superficial covering with sand and gravel.In the area, the chemical pollution is a mixed one, with crude oil and salted water, currently salted water pollution gives the prominent character of the area. For the studied location, is recommended the use of grassland with the application of overseeding and an organic and mineral ameliorative fertilization, after performing the territory improvement (Toti et al., 1999).

## CONCLUSIONS

Currently, oil pollution is reduced, hydrocarbons from the soil are in continuous degradation and after a suitable ameliorative work, will no longer be a danger.

Soil salinisation, predominant in territory, was the main and the most severely limiting factor in fertility reducing or disappearance of microorganisms development.

To apply the improvement methodology (Dumitru M. et al., 2005) , which will remove soluble salts and oil residues from soil and water of Strimbu river, is necessary that

before applying agropedoamelorative measures that will remove pollution, to apply measures of physical remediation. This, on one hand releases land for other measures, stops accidental pollution and exclude the possibility that Strimbu river continue to be polluted (all discharge pipes have to be removed or blocked).

For execution of improvement works of this polluted soil, the following steps are still needed: further surveying measurements showing the areas affected by erosion (erosion depth), longitudinal and transversal profiles trough the formations; geotechnical, hydrological and hydro-geological studies showing land stratification, geotechnical characteristics, groundwater level, evaluation of surface water flow through the associated tank (figure 2).

Figure 2. Gully erosion (Original).

Next, a feasibility study will be carried out, that will determine the design solution, the necessary work, related costs. By the end of the project can be made a stripping of the artificial roads for 30cm, a stripping of 30cm for the areas with gaps produced by salinisation and waste oil products; collection of gravel waste, concrete, scaffolds; removing of uncovered underground pipes that are not used or cannot be used.

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# THE EVOLUTION OF TOTAL HETEROTROPHIC BACTERIA IN SOIL POLLUTED WITH CRUDE OIL

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***Keywords****: soil pollution, crude oil, microbiology, total heterotrophic bacteria (THB)*

## ABSTRACT

*Bioremediation is defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. It is an attractive process due to its low cost and the benefit of contaminant/pollutant mineralization into $CO_2$ and $H_2O$. The goal of bioremediation is to degrade organic contaminants/pollutants to concentrations that are undetectable, or if detectable, to concentrations below the limits established as safe or acceptable by regulatory agencies.Petroleum hydrocarbons are used by microorganisms as a source of carbon and energy. The most rapid and complete degradation of the majority of pollutants is brought about under aerobic conditions. In this paper are presented the results concerning the bioremediation of polluted soils with crude oil and the evolution of total heterotrophic bacteria. It was used a natural hydrocarbon absorbent product and a bacterial inoculum to accelerate the naturally occurring biodegradation of petroleum hydrocarbons.*

## INTRODUCTION

Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention. The interest in environmental pollution has increased for the entire population of the globe. Various institutions and organizations, some multidisciplinary other specialized publications, focused solely on pollution issues. There is no life without soil (Pepper et all, 1996; Alexander, 1997).Many developing countries face serious problems with soil pollution, but environmental concerns seem to be a luxury given the economic situation in most countries of our days. Furthermore, soil structure and biology can be dramatically disturbed or even destroyed making the land useless for agricultural purposes (Lee and Levy, 1989). Crude oil is a complex mixture of hydrocarbons. It includes a saturate fraction, an aromatic fraction, asphaltenes, and resins (Atlas, 1992; Okoh and Trejo-Hernandez, 2006). Due to this complexity, petroleum hydrocarbons cannot be fully degraded by a single strain of microorganisms but its decomposition is achieved by microbial consortia and their broad enzymatic capacity (Norris, and Matthews, 1994).There are many genera of known oil-degrading microorganisms, including bacteria such as *Achromobacter, Acinetobacter, Actinomyces, Bacillus, Microbacterium, Pseudomonas, Streptomyces* and *Vibrio*, and fungi or yeast such as *Allescheria, Aspergillus, Candida, Debayomyces, Penicillium, Saccharomyces* and *Trichoderma*. Under natural conditions,

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these microorganisms in most areas comprise very few, compared with the total number of identified microorganisms. However, at petroleum hydrocarbon polluted soils, these populations may grow and increase because they use petroleum hydrocarbon as a carbon source (Alexander, 1997; Voiculescu et all, 2003).

## MATERIALS AND METHODS

The main objective of this research is testing the natural hydrocarbon absorbent named ECOSOL. It is tested the capacity to increase the biodegradation of petroleum hydrocarbons by stimulating the bacteria. To achieve data concerning the bioremediation of polluted soil with petroleum hydrocarbons was realized a greenhouse experiment. The soil used for this experiment (calcic chernozems) was reaped from arable layer 0-20 cm (Teleorman). This type of soil was chosen because of its currency in our country, also, for its physical, chemical and biological properties favorable to plant growth.The study focused on the application of the two major technologies known in bioremediation method such as: soil biostimulation based on environmental conditions improvement for microorganisms multiplication and activity to degrade petroleum hydrocarbons, and bioaugmentation based on enriching the soil with specific biodegrading hydrocarbons microorganisms. Biostimulation - the first technological link included a innovation element based on using an organic compound made from cellulose fibers for soil polluted conditioning with additives to optimize its structure, water and air circulation regime in soil, and not least achieving a protective interface between degrading microorganisms and pollutant. Ecosol compound was chosen for experiment by analysing a series of organic compounds suitable for conditioning soil contaminated with organic pollutants, especially because of its biodegradability properties.Bioaugmentation - the second technology link was achieved by soil inoculation with bacterial bioproducts made from specific bacteria selected and tested in the laboratory for their ability to degrade petroleum hydrocarbons.The experiment was set up by artificial pollution of a cambic chernozem with different quantities of ECOSOL. After 21 days from pollution, the soil was inoculated with bacteria. The bacterial inoculum was developed from microorganisms that occur naturally in the soil like *Pseudomonas, Mycobacterium, Arthrobacter globiformis* and *Bacillus megaterium*.ECOSOL is an absorbent natural product, meant to facilitate quick and efficient biodegradation of hydrocarbons from contaminated soils. Accelerates biostimulation and favors the development of existing bacteria from the soil, with strong effects in crude oil biodegradation. This natural biodegradable product is obtained from vegetal fibers from celluloid waste, all treated and with additives, being used in order to bring soils back to normal fertility levels.

The experimental variants are:

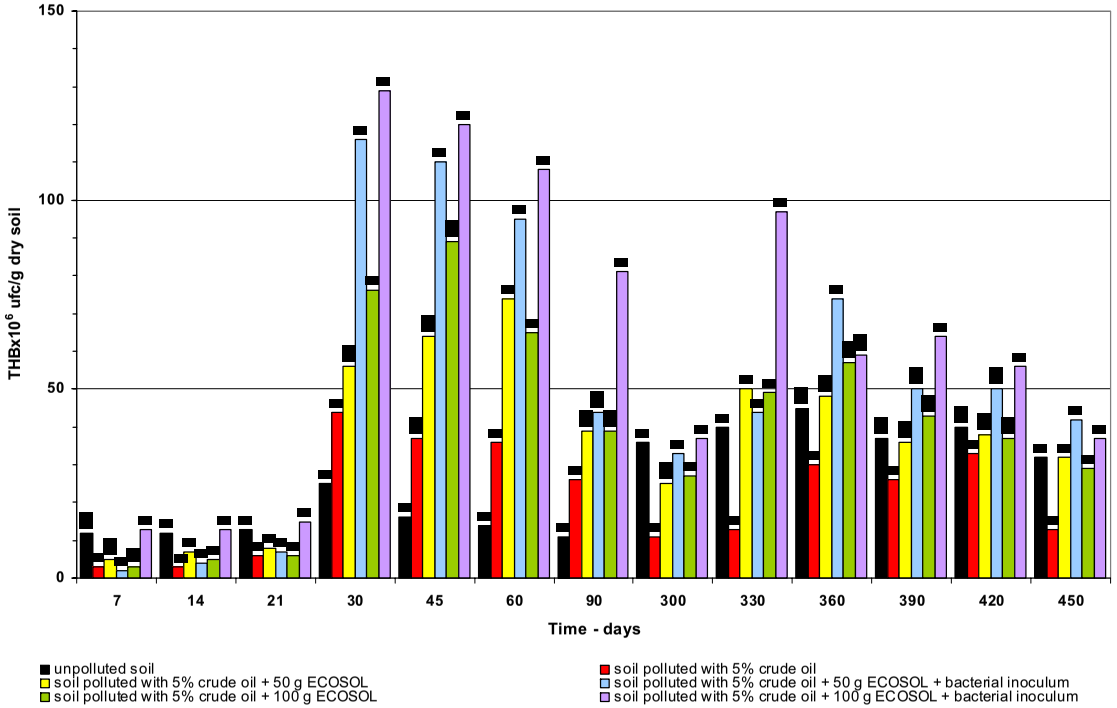
- ✓ $V_1$, control (unpolluted soil);
- ✓ $V_2$, polluted soil with 5% crude oil;
- ✓ $V_3$, polluted soil with 10% crude oil;
- ✓ $V_4$, polluted soil with 5% crude oil + 50 g ECOSOL;
- ✓ $V_5$, polluted soil with 5% crude oil + 50 g ECOSOL + bacterial inoculum;
- ✓ $V_6$, polluted soil with 5% crude oil + 100 g ECOSOL;
- ✓ $V_7$, polluted soil with 5% crude oil + 100 g ECOSOL + bacterial inoculum;

- ✓ $V_8$, polluted soil with 10% crude oil + 100 g ECOSOL;
- ✓ $V_9$, polluted soil with 10% crude oil + 100 g ECOSOL + bacterial inoculum;
- ✓ $V_{10}$, polluted soil with 10% crude oil + 200 g ECOSOL;
- ✓ $V_{11}$, polluted soil with 10% crude oil + 200 g ECOSOL + bacterial inoculum.

The values obtained by analyzing soil and plant samples were processed using more specific methods of mathematical statistics. Analysis of variance for establishing Fischer and Tukey tests determined for $\alpha = 0.05$, which shows the changes produced on soil and plant characteristics, the effects of treatments applied. ANOVA method provides information allowing the calculation of limit differences used in multiple comparison methods and the mean average for each graduation of studied factor. By correlation method was determined the linear correlation coefficient or the correlation ratio (index), for assessing the intensity of the relationship between variables. For the estimation of a link between the two characteristics studied, stochastic experiments were conducted by achieving regression equations.

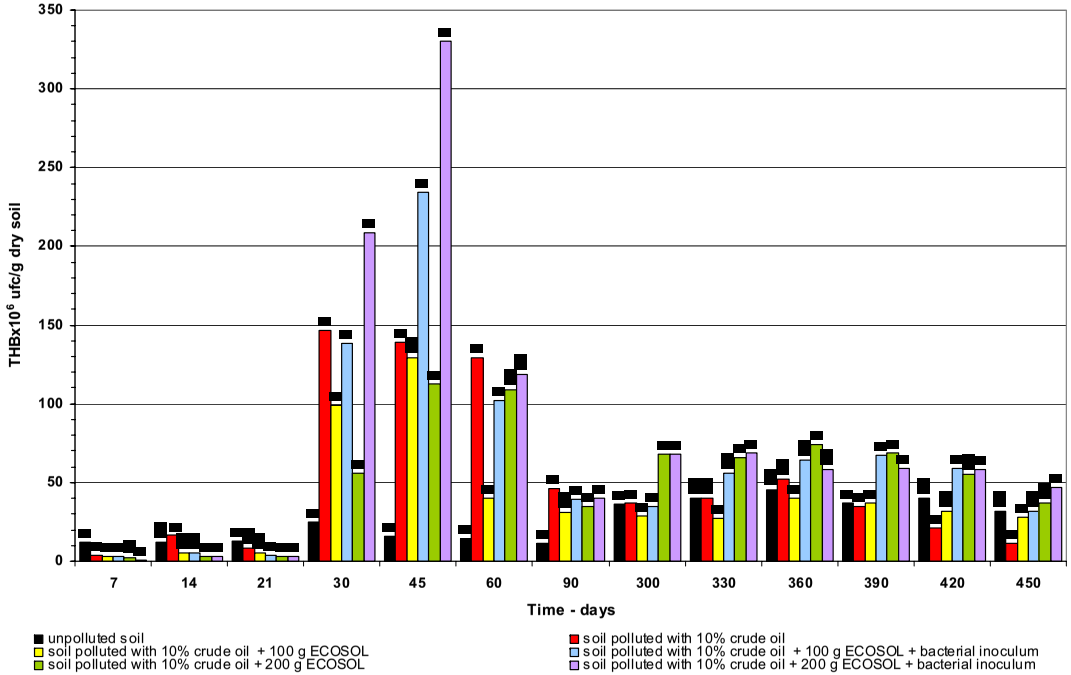
## RESULTS AND DISCUSSIONS

Bacterial inoculum application after 21 days from the experiment beginning was reflected in very significant increases in the levels of total heterotrophic bacteria (THB) determined in polluted soil.Immediately after controlled soil pollution with crude oil, at impact, bacterial populations have decreased the multiplication rate, and later, after an adaptation and selection process, the resistance component will extensively proliferate. For soil polluted with 5% crude oil, the bacterial top, including those placed in the soil by inoculation was 30 days after impact, respectively 7 days after inoculum application, while in soil polluted with 10% crude oil, multiplication top of bacteria was observed in the determination made at 45 days after impact and 21 days after inoculum application, showing once again how necessary is for microorganisms habituation and adaptation to environment being a function of pollutant concentration.In soil excessively polluted with crude oil, bacterial population size in conditioned variant with Ecosol maximum dose (1%) presented values comparable to those of inoculated variants, demonstrating the protective and stimulation effect of soil bacteria, including those involved in the degradation of petroleum hydrocarbons exercised by organic compound applied Ecosol.The quantitative evolution of bacterial communities from soil polluted with crude oil clearly demonstrated that the Ecosol application decreases the time needed for microorganisms involved in petroleum hydrocarbons biodegradation to adapt at substrate and conditions of pollution. Frequent bacterial isolated from experimental variants, in most determination stages were: *Pseudomonas, Arthrobacter, Mycobacterium*, bacterial of the most reputable in terms of species with high capacities in hydrocarbon biodegradation.Isolates of *Pseudomonas*, *Arthrobacter* and *Mycobacterium* led to the establishment of inoculum applied to the soil, and very high frequency showed a better survival rate and their adaptability to environmental conditions. Bacterial isolated as *Pseudomonas* and *Arthrobacter* dominated the heterotrophic bacteria population in the majority of experimental variants, occasionally accompanied by species belonging to the genera: *Micrococcus*, *Enterobacter* and *Flavobacterium*. Dynamics of microorganisms inoculated showed a slight adaptability in soil polluted with 5% crude oil, but complete inhibition in the first 30 days of experiment at 10% crude oil. After the acclimatization period by 30 days, the excessive concentration of pollutants has been massive multiplication of bacteria in inoculated variants, especially in the conditioned variant with Ecosol maximum dose. At 45 days, there was a real explosion in variants of bacteria inoculated with THB values double, triple compared with uninoculated variants.




*for each moment of TPH deteremination the experimental variants were marked with the same letter (a, b, ...) do not differ significant (test Tukey - $\alpha$ = 0,05)

Figure 1 The evolution of total heterotrophic bacteria (THB) in soil polluted with 5% crude oil in different experimental variants – analysis of variance




*for each moment of TPH deteremination the experimental variants were marked with the same letter (a, b, ...) do not differ significant (test Tukey - $\alpha$ = 0,05)

Figure 2 The evolution of total heterotrophic bacteria (THB) in soil polluted with 10% crude oil in different experimental variants – analysis of variance

At 60 days, bacterial populations have begun to reduce their size, as the value of soil polluted with 10% crude oil and untreated. This demonstrates that the application reduces the time needed to adapt to Ecosol substrate for microorganisms involved in petroleum hydrocarbons biodegradation.The value of the total heterotrophic bacteria (THB) in soil is a response to a large number of factors, including crude oil content, which causes new ecological conditions with a strong impact on microbial evolution. These conditions affect not only the size, but also the diversity of bacterial communities. The soil is mainly colonized by bacterial genera species able to use hydrocarbons from crude oil in its metabolism (Voiculescu et al., 2003).Survival of microorganisms in petroleum hydrocarbons medium in uninoculated variants and after bacterial inoculation in the inoculated variants is a key deciding factor in the rate of biodegradation of hydrocarbons in soils.

## CONCLUSIONS

For soil polluted with 5% crude oil, the bacterial top, including those placed in the soil by inoculation was 30 days after impact, respectively 7 days after inoculum application, while in soil polluted with 10% crude oil, multiplication top of bacteria was observed in the determination made at 45 days after impact and 21 days after inoculum application, showing once again how necessary is for microorganisms habituation and adaptation to environment being a function of pollutant concentration. The microorganisms inoculated showed a slight adaptability in soil polluted with 5% crude oil, but complete inhibition in the first 30 days of experiment at 10% crude oil. After the acclimatization period by 30 days, the excessive concentration of pollutants has been massive multiplication of bacteria in inoculated variants, especially in the conditioned variant with Ecosol maximum dose. The application reduces the time needed to adapt to Ecosol substrate for microorganisms involved in petroleum hydrocarbons biodegradation.

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# CONSIDERATIONS UPON THE CLIMATIC CHARACTERISTICS OF THE AGRICULTURAL YEAR 2011-2012 IN THE SOUTH-WEST OF ROMANIA

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***Key words*:** *drought, scorching heat, frost, thermal and hydric stress.*

**ABSTRACT**

*The atypical weather during 2011 has also continued in 2012 (Marinică & all, 2011). Consequently, the winter of 2011, in Oltenia had two distinct parts: a first warmish and droughty part, and a second cold part, marked by drought too. In November, the weather was colder than usual. This was the first excessively droughty autumn of the last 13 autumns in Oltenia. During the winter of 2011-2012, although most of it was warm, there were registered 20 days of excessive frost and abundant snowfalls (25 January 2012-15 February 2012). In April 2012 the drought continued. The excessively warmish weather returned in summer, associated with an intense drought. On the whole the agricultural year 2011-2012 was an excessively droughty year (ED), and the excessive drought was mainly registered in two essential periods: in autumn and during the whole summer, namely in the periods in which there is a maximum need of water. The paper analyses in detail the characteristics of this agricultural year and the consequences on crops.*

## INTRODUCTION

In this time interval, the crops bears significant thermal variations from the cold and frost of winter to the heat, scorching heat and sun glow of summer. Hydrically, the variations are extremely high from the drought period in the first part of autumn (minimum pluviometric) to the rainy period in the second part of autumn (secondary maximum pluviometric), then the solid and mixed precipitations during winter, the main maximum pluviometric during spring and first part of summer and in the last part of summer the droughty period usually returns. From germination and emergence up to harvest, during all vegetation stages phenomena of thermal and hydric stress occur, representing critical period for plants, caused by natural climatic variations (Sandu & all 2010). We will further analyze the climatic and agroclimatic aspects of the agricultural year 2011-2012.

## MATERIALS AND METHODS

For our research on this interval of analysis we used the data from Oltenia MRC Archive, NAM Bucharest (National Administration of Meteorology), maps drafted by the

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Laboratory of Agrometeorology (NAM), synoptic maps, data provided by the archive of satellite and radar images , as well as the facilities provided by Office.

## RESULTS AND DISCUSSIONS

**The warmish and droughty autumn of 2011.**

***The thermal regime of the autumn of 2011.*** The seasonal average values were comprised between 9.1°C in Voineasa in the submountainous area and 12.6°C in the south-west and west of Oltenia Dr. Tr. Severin and Calafat. Their deviations from the multiannual means calculated for the interval 1901 – 1990 were comprised between -0.9°C in Slatina and +1.9°C in Voineasa. The classification of thermal time type for this autumn, according to Hellmann Criterion were comprised between normal (N) in most of the region and warm (W) in Voineasa. The seasonal mean calculated for the entire region was 10.8°C, and its deviation from the normal was 0.3°C which leads to the conclusion that the average autumn was thermally normal (N). However, September imposes itself as a warm month (W) for the entire region, according to the monthly means and their deviations.

***The thermal regime of September 2011.*** *The monthly temperature means* were comprised between 18.5°C in Bâcleş and 22.8°C in Calafat, and their deviations from the monthly multiannual temperature means were comprised between 0.9°C in Slatina and 9.3°C in Voineasa, designating a thermal regime comprised between normal (N) in Slatina and very warm (VW) in Băileşti, Tg. Logreşti, Polovragi, Voineasa, Parâng and Ob. Lotrului.

*The general mean deviation* for the entire region was 3.9°C, which designates a warm month (W) for the entire region. ***All days of September were summer days. Of all these a number of 18 days were tropical days, namely they had daily maximum thermal values ≥ 30°C.***

***The pluviometric regime of the autumn of 2011.*** *The seasonal sums of precipitations* registered in the autumn of 2011 were comprised between 14.0 l/m$^2$ in Bâcleş in Mehedinţiului Hills and 56.2 l/m$^2$ in Bechet in the south of Oltenia Plain. The percentage deviations of these deviations from the multiannual means were comprised between -90.3% in Bâcleş and -58.3% in Bechet which leads to a classification of the pluviometric time type at the meteorological stations in Oltenia as exceptionally droughty (ED) in the whole region, an aspect confirmed also by the general mean for the entire region of 33.7 l/m$^2$ and its percentage deviation of -78.7%. The smallest values of temperatures were registered in November 2011 with values of 0 l/m$^2$ in most part Oltenia Plain and with a general mean for the entire region of 1.1 l/m$^2$, being the ***droughtiest month registered after 1950,*** constituting a climatic record.

In the end of autumn, on 30 November 2011, in Oltenia the moderate drought (MD) was present on extended areas in the south-west, north-east of the region (*humidity reserve in the soil layer 0-50 cm between 300 and 600 m$^3$/ha*) and in the most part of the region was satisfactory (SV).

**Winter of 2011-2012.** ***The thermal regime of the winter of 2011-2012.*** The seasonal temperature means were comprised between -2.5°C in the Subcarpathian Depressions area in Apa Neagră and 0°C in Dr. Tr. Severin in the west of the region. Their deviations from the multiannual means were comprised between -1.5°C in Apa Neagră and -0.1°C in Bâcleş, and according to Hellmann criterion the classification of thermal time type in this winter were comprised between cool (CO) in Bechet, Slatina, Apa Neagră and Parâng and normal (N) in most part of the region. December and January were warmish (WS) month, but February especially in the interval 1-15 February was excessively cold.

***The thermal regime of February 2012.*** *The monthly average temperature values* in the air were comprised between -6.6°C in Caracal in the south-east of Oltenia and -3.9°C in Rm. Vâlcea in Olt Couloir, and their deviations from the multiannual means were comprised between -6.2°C in Bechet in the south of the region and -2.7°C in Voineasa, in the submountainous area. According to Hellmann criterion, the thermal time type in Oltenia were comprised between very cold (VC) in most part of Oltenia Plain in Tg. Logreşti and in the Subcarpathian Depression Apa Neagră and cold (CL) in the high hills and mountainous area.

*The minimum temperature values* in the air were comprised between -28.9°C in Băileşti and -17.9°C registered in Drăgăşani both being registered on 1 February, and the monthly minimum temperatures mean was -23.1°C, lower than in January. *The minimum temperature values* in Calafat (-26.1°C), Bechet (-24.0°C), Băileşti (-28.9°C) Tg. Logreşti (-28.1°C), Apa Neagră (-28.4°C) and Obârşia Lotrului (-28.6°C), constituting *climatic records* for these stations, being the lowest of all the existent data.

*The minimum temperature values at the soil surface* were extremely low and were ranged between -23°C in Rm. Vâlcea and -32.2°C in Tg. Logreşti, registered on 9 February, and at some meteorological stations on 1 and 2 February.

***The thermal regime of the winter of 2011-2012.*** The seasonal precipitations values in this winter were comprised between 123.2 l/m$^2$ in Bechet and 232.9 l/m$^2$ in Apa Neagră, and their percentage deviations from the normal values were extremely diverse and were ranged between -19.2% in Polovragi in the subcarpathian depressions area and 70.2 l/m$^2$ in Craiova in the central part of the region leading to a classification of pluviometric time types from droughty (D) in Polovragi and excessively rainy in Craiova. The general mean for the entire region was 148.8 l/m$^2$, and its percentage deviation from the normal general mean was of 8.5% which designates a pluviometric normal winter (N). The good pluviometric situation is due to the interval 24 January-10 February which was excessively rainy. December 2011 was excessively droughty (ED). January was excessively rainy also due to the aforementioned interval. February was very rainy (VR). In the end of winter, on 30 January *water reserve in the soil layer of 0-100 cm* was almost optimum (ApO) in most part of the region and optimum in the central part of the region.

**Spring of 2012.** ***The thermal regime of the spring of 2012.*** The seasonal temperature means in this spring were comprised between 9.1°C in Voineasa and 14.2°C in Băileşti in Oltenia Plain. Their deviations from the multiannual means were comprised between 1.3°C in Slatina and 2.7°C in Apa Neagră which leads to a classification of thermal time types from warm (W) in most part of the region to very warm (VW) in Băileşti and Drăgăşani. In detail, March and April were warm (W), and more warmish (WS) on average and normal (N) in the plain area. In March monthly maximum temperature values were registered in the last decade and were comprised between 21.3°C in Polovragi and 26.3°C in Bechet. There were registered two days with maximum thermal values ≥ 25°C (summer days) on 18 and 19 March. *In April the monthly maximum temperature values* were registered in the last five days of the month and were ranged between 26.6°C in Bâcleş and 29.3°C in Dr. Tr. Severin. In May the *monthly maximum temperature values* in the air were comprised between 28.1°C in Polovragi and 33.0°C in Calafat, and most of them were registered on 12.

***The pluviometric regime of the spring of 2012.*** *The seasonal temperature means* were comprised between 121.9 l/m$^2$ in Caracal in Romanaţilor Plain and 461.4 l/m$^2$ in Polovragi in the subcarpathian depressions area. The deviations of these quantities from the normal values were comprised between -20.5 l/m$^2$ in Voineasa and 236.2 l/m$^2$ in Polovragi. We notice only two negative deviations: in Voineasa and in Caracal in Romanaţilor Plain (-20.3 l/m$^2$). The general mean for the entire region was 237.4 l/m$^2$, designating the spring

of 2012 as the rainiest season of this agricultural year. The percentage deviations were ranged between -14.3% in Caracal and 104.9% in Polovragi, which leads to a classification of pluviometric time types from little droughty (LD) in Caracal and Voineasa and exceptionally rainy in Craiova, TG. Logreşti and Polovragi. The deviation of the general mean from the normal was of 58.9 l/m$^2$, which leads to the classification of a very rainy spring (VR). March is an excessively droughty (ED) month in Oltenia area, April a very rainy (VR) month and May excessively rainy (ER) in all Oltenia with a general mean of 149.6 l/m$^2$ and a deviation from the normal of 69.6 l/m$^2$ (86.9%).

In the end of May (30 May 2012), *the water soil reserve* which is accessible to the autumn wheat plants *on the depth of 0-100 cm* was optimum (O) in the north of the region, almost optimum (AO) in the median part of the region and almost satisfactory (AS) in the south-east of the region.

On the *soil depth of 0-50 cm*, on the same date, the water soil reserve in the corn crop was excessive (E) in the north-west of the region, optimum (O) in the north-east of the region and extreme south (Bechet area) and almost optimum (AO) in the southern half, and almost satisfactory (AS) in the extreme south-east of Romanaţiului Plain.

**Scorching summer of 2012.** ***The thermal regime of the summer of 2012.*** The seasonal monthly means of the air temperature were comprised between 19.1°C in Voineasa and 26.6°C in Băileşti, and the deviations from the normal means were comprised between 2.9°C in Voineasa and 5.0°C in Apa Neagră which leads to a classification of the thermal time types according to Hellmann criterion as a very warmish summer (VW) for all the meteorological stations from Oltenia. *The seasonal general mean for the entire region* was 23.8°C, and its deviation from the normal was 3.7°C which confirms that the summer of 2012 was very warmish (VW) for the entire region. *The number of scorching days* was comprised between 4 in Voineasa and 36 in Calafat and Bechet, in Oltenia Plain where 25.6% of the summer days were scorching. *The number of scorching heat days* was comprised between 21 in Voineasa in the submountainous area and 63 in Calafat and Bechet in Oltenia Plain where 68.5% were scorching heat days, which shows the high duration and intensity of the thermal stress on crops.

***The pluviometric regime in the winter of 2012.*** *The seasonal quantities of precipitations* were comprised between 44.0 l/m$^2$ in Caracal and 207.3 l/m$^2$ in Apa Neagră, and their deviations from the multiannual means were comprised between -73.7% in Caracal in Romanaţilor Plain and -10.6% in Apa Neagră. There was one exception in Bâcleş in Mehedinţiului Hills where it was 28.1% due to the local conditions which favored some significant showers on a very restricted area, which leads to a classification of pluviometric time types in the summer of 2012 from very droughty (VD) to exceptionally droughty (ED) in most part of the region (81.3% of the territory). There is one exception in Bâcleş where the summer was very rainy (VR), but on a restricted area. The drought intensity was small in the areas of Rm. Vâlcea and Apa Neagră where it was droughty (D) and little droughty (LD) due to the conditional local too. *The water reserve in the end of August* in the unirrigated corn crop, on the soil depth of 0-100 cm registered low and extremely low values, the pedological drought being moderate (MD), severe (SD) and extreme (ED), on almost all agricultural territory of the country, and in Oltenia the extreme drought (ED) and severe drought (SD) was extended in all the region, excepting the north-east of Vâlcea county where the drought was moderate (MD).

***The air temperature regime in the summer of 2012.*** *The seasonal air temperature means* were comprised between 19.1°C in Voineasa in the submountainous area and 26.6°C in Băileşti in Oltenia Plain, and their deviations from the multiannual means were comprised between 2.9°C in Voineasa and 5.0°C in Apa Neagră, which leads, according to

Hellmann criterion to a classification of a very warmish summer (VW) in most part of the region with one exception in Apa Neagră area where the summer was excessively warmish (EW). On the whole, the summer of 2012 was very warmish (VW), an aspect also confirmed by the deviation of the general mean from the normal value of 3.7°C. *The number of scorching days* was comprised between 4 in Voineasa and 36 in Calafat and Bechet, in Oltenia Plain where 25.6% of the summer days were scorching. *The number of scorching heat days* was comprised between 21 in Voineasa in the submountainous area and 63 in Calafat and Bechet in Oltenia Plain where 68.5% were scorching heat days, which shows the high duration and intensity of the thermal stress on crops.

**The annual precipitations regime during the agricultural year of 2011-2012.**

*The annual precipitations sums* registered in this agricultural year were comprised between 340.8 l/m$^2$ in Caracal in Romanaților Plain and 761.4 l/m$^2$ in Apa Neagră in the subcarpathian depressions area, and the *quantitative deviations* from the multiannual means calculated for the interval 1901-1990 were comprised between -347.9 l/m$^2$ in Voineasa in the submountainous area and -46.4 l/m$^2$ in Craiova in the central part of Oltenia. The percentage deviation from the normal values were comprised between -44.9% in Voineasa and -8.7% in Craiova, which according to Hellmann criterion leads to a classification of an exceptionally droughty year in most part of the region excepting three restricted areas: Craiova where it was little droughty (LD) and Tg. Logreşti and Rm. Vâlcea where it was droughty (D) due to the local conditions that, in some situations, caused some abundant precipitations.

*The precipitations mean for the entire region* was 519.8 l/m$^2$ and its percentage deviation from the normal value[5] was -23.4%, which confirms the classification of excessively droughty (ED) agricultural year. We conclude that ***on the whole the agricultural year 2011-2012 was an excessively droughty year (ED), and the excessive drought was mainly registered in two essential periods: in autumn with the result that it prevents agricultural works from being carried out and crops from being set up in the optimum time and during all summer, namely in the periods in which there is a maximum need of water***. Drought was associated with high temperatures, sun glow and scorching heat during extended periods of time.

## CONCLUSIONS

***On the whole of the agricultural year, 2011-2012 was an excessively droughty year (ED), and the excessive drought was mainly registered in two essential periods: in autumn with the result that it prevents agricultural works from being carried out and crops from being set up in the optimum time and during all summer, namely in the periods in which there is a maximum need of water.*** Drought was associated with high temperatures, sun glow and scorching heat during extended periods of time.

*The droughty pluviometric time type (DT)*) predominated 63.5% of the year and the region area, the rainy time (RT) 38.6% of the year and the region area, and the normal time (NT) only 6.2% (fig. 1). During all year, the *thermal stress* have been intense not only in autumn, but also in summer and winter in the interval 25 January-15 February, the temperatures falling under the plants' critical thresholds of resistance to frost. As a consequences of the excessive droughty *the degree of crops damage* was high in all the country and especially in the extracarpathian area, not only in Oltenia.

[5] ***The multiannual means*** for the interval of 90 years 1901-1990 are considered ***normal values***.

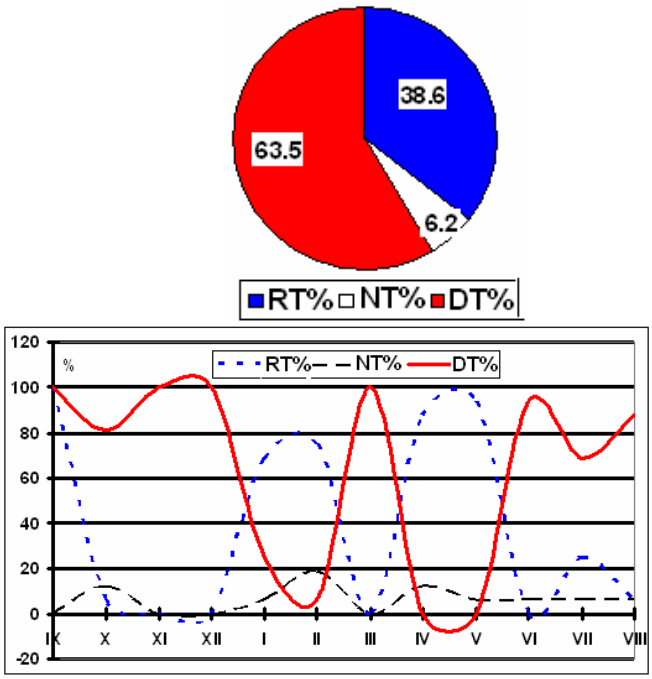


Fig. no 1, Space-time extension of the pluviometric time types the agricultural year 2011-2012.

The Romanian Government decided to help the countrymen and farmers by drafting GD 897/2012, consisting in awarding financial aids for the drought destructive effects. *The losses of wheat crop* in the entire country were 600 thousands of tones les compared to the mean of the last years. The losses of *sunflower crops* were about 40-45% of production, and in what the oil content is concerned, the reduction was about 3-4%.

The losses of *vegetables-fruits* were about 40%. The producers' losses, because of the damages caused by the drought, were 2,5 billions of euros or more, and the aid to hectare given by the Ministry of Agriculture represented a recovery of only hundreds of millions of euros. All these led to the *raise in prices of agricultural products and of the cost of living in general,* especially given that there have been registered damages in the domain of electricity generation too (Hidroelectrica company registered losses of 253 million of euros or more), and the electricity consumption during summer was high mainly as a consequence of the use of air conditioning.

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***Seria:*** ✓ *Biologie*
✓ *Horticultură*
✓ *Tehnologia prelucrării produselor agricole*
✓ *Ingineria mediului*

**Vol. XVII ( LIII ) - 2012**

# PRELIMINARY STUDIES ON THE NATURAL HABITATS FROM BUILA-VÂNTURARIȚA MOUNTAINS

Neblea Monica[1], Bodescu F.[2]

***Key words*****:** *natural habitats, Buila-Vânturarița Mountains, Romania*

## ABSTRACT

*This paper presents the description of two natural habitats from Buila-Vânturarița Mountains: 6170 Alpine and subalpine calcareous grasslands and 4060 Alpine and Boreal heaths. These habitats were presented using following diagnosis elements: code and name, correspondence with CORINE, EMERALD, PALAEARCTIC HABITATS, EUNIS and ROMANIAN classification, correspondence with plant associations, distribution in territory, site description, structure of the community and conservative value.*

## INTRODUCTION

Buila-Vânturarița Massif is located in the southern part of the Lotru Valley, being bounded on the east by Olăneşti Valley and Bistrita Valley on the west. The limestone ridge has a length of 14 km and contains the highest peaks: Vioreanu (1866 m), Buila (1849 m) and Vânturarița Mare (1885 m). It belongs to the Vâlcea County from the administratively point of view.

The Buila-Vânturarița Mountains are constituted by metamorphic and sedimentary deposits (sedimentary of the Lotru Mountains). The metamorphic rocks outcrop beneath the eroded sedimentary deposits (Curmătura Builei) in the northwestern slopes, the river valleys of Bistrița, Costeşti, Cheia, Olăneşti, and in the ridge area. The sedimentary sequence comprises Upper Jurassic and Lower Cretaceous deposits.

The predominant soils are rendsinas. The acid brown forest, humic-feriilluvial podzolic and criptopodzolic soils, with acid reaction and different degrees of podzolization are frequently in the inferior and superior mountain level. Along the valleys alluvial soils are developed.

The climate is vertically leveled, according to the relief, with variations and inversions of climate from a slope to another. The annualy average temperatures are 1-9$^0$C, while rainfalls recorded between 600-900 mm/year values, in the inferior level and over 900 mm/year in the subalpine level. The dominant winds blow from NV-SE direction.

Buila-Vânturarița Mountains represent a less studied area, in the scientific literature being mentioned research of Buia M. et Păun M., 1956; Păun M. et Popescu Gh., 1971; Păun M. et Popescu Gh., 1978; Popescu Gh. et al. 2003. The present paper

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contributes to the enrichment of the phytocoenological information in this territory, which in 2004 was declared the National Park Buila-Vânturarița, the future site of the NATURA 2000 Network. It is the smallest national park from Romania, with an area of 4186 ha.

## MATERIAL AND METHODS

Research concerning the inventory of the natural habitats were performed in 2012 as part of the project SOP-Environment-*The assessment conservation and detailed mapping of habitats (forest habitats, grasslands/meadows, screes) from the Buila-Vânturarița National Park.*

There are established and crossed several routes to Pătrunsa Hermitage, Curmătura Builei, Cacova, Buila Peak, Vânturarița Peak. We used following diagnosis elements to characterize the natural habitats: code and name, correspondence with CORINE, EMERALD, PALAEARCTIC HABITATS, EUNIS and ROMANIAN classification, correspondence with plant associations, distribution in territory, site description, structure of the community and conservative value.

For the characterization of the natural habitats have been used works of synthetis of some authors as follows: Ciocârlan V., 2009; Coldea Gh., 1991; Doniță et al., 2005; Neblea, 2011; Oltean M. et al., 1994; Sanda V. et al., 2003; Gafta D. et Mountford O., 2008.

## RESULTS AND DISCUSSIONS

Phytocoenological investigations of the Buila-Vânturarița Mountains allowed the identification and analysis of 14 natural habitats according to the Natura 2000 classification, which will be characterized the 6170 Alpine and subalpine calcareous grasslands and 4060 Alpine and Boreal heaths.

The analyzed habitats are situated in the mountain and subalpine level. The conservation state is good, but the potential threats are: increasing of grazing, wind deflation, flora collection by the tourists and sheperds.

**6170 Alpine and subalpine calcareous grasslands**

**Correspondence**:

EMERALD: -

CORINE: 36.4 Alpine and subalpine calciphilous grasslands; 36.43 Stepped and garland grassland

PAL. HAB. 1999: 36.43922 East Carpathian *Festuca versicolor* grasslands; 36.43921 East Carpathian sesleria-evergreen sedge grasslands

EUNIS: E4.4392 East Carpathian calciphile stepped grasslands

R3605 South-Eastern Carpathian grasslands with *Festuca versicolor* and *Sesleria rigida* ssp. *haynaldiana*; R3612 South-Eastern Carpathian grasslands with *Carex sempervirens* and *Sesleria bielzii*; R3611 South-Eastern Carpathian grasslands with *Sesleria rigida* ssp. *haynaldiana* and *Carex sempervirens*

**Plant associations**: *Seslerio-Festucetum versicoloris* Beldie 1967; *Seslerio-Caricetum sempervirentis* Puşcaru et al. 1956; *Seslerio haynaldianae-Saxifragetum rochelianae* Boşcaiu 1971.

**Distribution in territory**: from Pătrunsa Hermitage to Curmătura Builei, Curmătura Builei, Cheia Gorges, Cacova, Buila Peak.

**Site description**: This habitat has been identified on the rocks with western, south-western, northern exposition at 1000 m to over 1700 m altitudes. It is developed on slopes with varying degrees of inclination 45-85$^0$, on rendzinic soils with limestone substrate (fig. 1). With

regard to the ecological preferences, these coenoses are xero-mesophilous, micro-mesotherms and in terms of soil reaction, are low acid neutrophilous.

**Community structure**:

*Sesleria rigida* ssp. *haynaldiana* achieves the highest coverage (70%) in the *Seslerio-Festucetum versicoloris* Beldie 1967 association. The plant grouping with *Sesleria bielzii* and *Carex sempervirens* dominates the calcareous rocks over 1500 m. In this coenoses, the highest coverage is realized by *Carex sempervirens* (70%). The phytocoenoses with *Saxifraga marginata* were identified at Curmătura Builei, being interspersed among the *Seslerio-Caricetum sempervirentis* association. These prefer shady slopes with northern exposition and 80-90$^0$ inclination. The herbaceous layer is dominated by *Carex sempervirens, Sesleria rigida* ssp. *haynaldiana* and *Saxifraga marginata*.

The typical elements to the sesleriets were identified in the floristic composition of the alpine and subalpine calcareous grasslands habitat, such as: *Festuca versicolor, F. rupicola* ssp. *saxatilis, Dianthus spiculifolius, Bupleurum diversifolium*, *Athamantha turbith* ssp. *hungarica, Onobrychis transsilvanica, Carduus kerneri, Polygonum viviparum, Ranunculus oreophilus, Anthyllis vulneraria* ssp. *alpestris, Saxifraga marginata, Poa molinerii, Campanula cochlearifolia, Aconitum anthora, Scrophularia heterophylla* ssp. *laciniata, Helianthemum oelandicum* ssp. *alpestre, Galium anisophyllon, G. album, Erysimum witmannii, Myosotis alpestris, Hieracium villosum, Euphrasia salisburgensis, Asperula capitata, Pedicularis comosa, Laserpitium latifolium, Thymus pulegioides, Pimpinella saxifraga, Daphne mezereum, Seseli libanotis, Cerastium alpinum, Alyssum repens, Scabiosa lucida, Minuartia verna, Gentiana verna*.

Also, are developed characteristic species to the chasmophytic vegetation from *Asplenietea* class, as follows: *Poa nemoralis, Cystopteris fragilis, Asplenium ruta-muraria, A. trichomanes, A. viride, Saxifraga paniculata, Cortusa matthioli*.

Fig. 1. Alpine and subalpine calcareous grasslands (Cheia Gorges)-Original

It is an endemic habitat, with high conservative value, that includes species of community interest: *Gentiana lutea*-Habitats Directive 92/43/EEC Annex Vb, OUG 57/2007-Annex 5Aa; *Iris aphylla*-Habitats Directive 92/43/EEC Annex IIb, OUG 57/2007-Annex 3b and *Campanula serrata*-Habitats Directive 92/43/EEC Annex IIb, OUG 57/2007 Annex 3b. Species as *Linum uninerve, Athamantha turbith* ssp. *hungarica, Dianthus spiculifolius, Onobrychis transsilvanica, Festuca versicolor, Saxifraga marginata, Sesleria bielzii* are sozological categories in Red List of superior plants from Romania (Oltean *et al*., 1994).

**4060 Alpine and Boreal heaths**

**Correspondence**:

EMERALD: 31.4 Alpine and Boreal heaths

CORINE: 31.43 Dwarf *Juniper* scrub; 31.491 High montane *Dryas* mats; 31.431 *Juniperus nana* scrub

PAL. HAB.: 31.4325 Carphato-Balkanic *Juniperus sabina* scrub; 31.49152 South-Eastern Carpathian *Dryas* mats; 31.431 Mountain *Juniperus nana* scrub

EUNIS: F2.2325 Carphato-Balkanic *Juniperus sabina* scrub; F2.2915 Carpatho-Balkanide *Dryas* mats; F2.231 Mountain *Juniperus nana* scrub

R3115 South-Eastern Carpathian scrub with *Juniperus sabina*; R3617 Scrub with *Dryas octopetala*; R3108 South-Eastern Carpathian scrub with *Juniperus sibirica*.

**Plant associations**: *Juniperetum sabinae* Csürös 1958; *Dryadetum octopetalae* Rübel 1911; *Campanulo abietinae-Juniperetum* Simon 1966

**Distribution in territory**: Costeşti Gorges, Stoguşoru Mountain, Cheia Gorges, Cacova, Buila Peak, Vânturariţa Peak.

**Site description**:

Communities with *Dryas octopetala* are stationed in subalpine level, on slopes with northern and western exposition, inclination degree of 30-60$^0$ and 1600-1800 m altitude. There are very well represented on the windy slopes from Buila Mountain. There are developed on rendzina, superficial soils, rich in humus, with low acid reaction.

Coenoses with *Juniperus sabina* populate sunny slopes, with southern exposition from mountain level (Costeşti Gorges, Stoguşoru Mountain, Cheia Gorges). It grows on limestone screes ensuring their stability (fig. 2).

*Juniperus communis* ssp. *alpina* forms compact groups on Vânturariţa Peak, while in Cacova or Buila Peak, we observed a penetration of them in grasslands where form clumps. Usually prefer the eastern sunny slopes, with rendzinic substrate and neutral-acid reaction.

The edapho-climatic conditions of this habitat favoured the development of the xero-mesophilous, micro-mesothermal and low acid neutrophilous species.

**Community structure:**

*Dryas octopetala* represents the main enlightening of the *Dryadetum octopetalae* Rübel 1911, with a coverage up to 75%. The floristic composition is completed by the characteristic species to sesleriets (*Dianthus spiculifolius, Carex sempervirens, Festuca rupicola* ssp. *saxatilis, Helianthemum oelandicum* ssp. *alpestre, Galium anisophyllon, Euphrasia salisburgensis, Bupleurum diversifolium*, *Polygonum viviparum, Anthyllis vulneraria* ssp. *alpestris, Minuartia verna, Sesleria bielzii*).

*Juniperus sabina* dominates the sabine communities and realizes a coverage of 50%, with whom vegetates as characteristic element *Rhamnus saxatilis* ssp. *tinctorius*. There are groupings edified by xero-mesophilous species, the thermophilic character is given by the taxons from *Quercetalia pubescentis* order (*Fraxinus ornus, Galium mollugo, Viola hirta, Peucedanum oreoselinum, Cornus mas*). We noticed a stratification of vegetation within groups of *Juniperus sabina* from the inferior mountain level (herbaceous,

shrub and tree layer ). The average height of the shrubs and trees layer is 4-5 m, with a canopy of 0,6-0,7. The tree and shrub layer consists of species as: *Juniperus sabina*, *Juniperus communis* ssp. *communis, Fraxinus excelsior, Corylus avellana, Acer campestre, Cornus sanguinea, C. mas, Tilia tomentosa, Clematis vitalba, Taxus baccata*.

Juniper communities from subalpine level are represented by *Juniperus communis* ssp. *alpina* (coverage of 80-90%). The group structure is edified by arcto-alpine and circumpolar elements. *Picea abies* and *Pinus mugo* appears in the shrub layer. The grass and subshrub layer is formed mainly of *Vaccinium myrtillus, V. vitis-idaea, Luzula luzuloides, Deschampsia caespitosa, Nardus stricta, Calamagrostis villosa*.

Floristic composition presents important species mentioned as rare, vulnerable, and non-threatened in Red List of superior plants from Romania (Oltean *et al*., 1994), such as: *Dianthus spiculifolius, Taxus baccata, Campanula grosekii, Thymus comosus, Veronica bachofenii, Linum uninerve, Juniperus sabina, Rhododendron myrtifolium*. Also, in these habitats vegetate *Campanula serrata* mentioned in Habitats Directive 92/43/EEC Annex IIb and OUG 57/2007 Annex 3b.

Fig. 2. *Juniperus sabina* communities (Costeşti Gorges) - Original

**Flora composition**: Edifying species: *Juniperus sabina, Dryas octopetala, Juniperus communis* ssp. *alpina, Campanula patula* ssp. *abietina*; Other important species: *Teucrium chamaedrys, T. montanum, Cornus mas, Fraxinus ornus, F. excelsior, Corylus avellana, Acer campestre, Cnidium silaifolium, Digitalis grandiflora, Asplenium trichomanes, A. ruta-muraria, Tilia tomentosa, Melica ciliata, Nepeta nuda* ssp. *nuda, Seseli libanotis, Elymus caninus, Molinia caerulea, Taxus baccata, Thymus comosus, Veronica bachofenii, Galium kitaibelianum, G. mollugo, Rhamnus saxatilis* ssp. *tinctorius, Viola hirta, Peucedanum oreoselinum, Linum uninerve, Campanula grosekii, C. sibirica, C. serrata, Allium fuscum, Verbascum glabratum, Calamintha sylvatica* ssp. *ascendens, Primula veris* ssp. *columnae, Dianthus spiculifolius, Bupleurum diversifolium, Polygonum viviparum, Rhododendron myrtifolium, Pinus mugo, Picea abies, Homogyne alpina,*

*Calamagrostis villosa, Vaccinium myrtillus, V. vitis-idaea, Oxalis acetosella, Deschampsia caespitosa, Luzula luzuloides, Nardus stricta, Carlina acaulis, Aconitum tauricum, Selaginella selaginoides, Carex sempervirens, Festuca rupicola* ssp. *saxatilis, Helianthemum oelandicum* ssp. *alpestre, Euphrasia salisburgensis, Anthylis vulneraria* ssp. *alpestris, Sesleria bielzii.*


## ACKNOLEDGEMENTS

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# *ARMERIA MARITIMA* SUBSP. *HALLERI* NEW SPECIES FROM CARPATHIAN CHAIN

Negrean G.[1], Ioana Ciortan[2]

***Keywords:*** *Armeria maritima subsp. halleri, european endemit, new species, Romania, Carpathian Chain, taxonomy, serpentine.*

## ABSTRACT

*During botanical research carried out in the Geopark Plateau Mehedinți, we found Armeria maritima L. subsp. halleri (Plumbaginaceae) on serpentine well represented in the Geopark.*

*From the 18 serpentine appearance of the whole Geopark Mehedinți, investigated until now, Armeria was found only where the altitude is more than 800 m.*

*Above 1898, in Romania, species was discovered by Grecescu in his journey to Godeanu Mountains by Țesna Valley, and since then has not been reported. Grecescu identified the plant as the Armeria elongata.*

## INTRODUCTION

The genus *Armeria* does not appear to be finally resolved taxonomically which is also true for its occurrence in different plant associations (BECKER and DIERSCHKE 2008).

Boissier (1848), in his monograph, recognized six species of *Armeria* in the Northwest and Center of Europa.

The first description our plant was known as *Armeria halleri* (basionym) by Karl Friedrich Wallroth în *Beitr. Bot.* 1: 150 (1800).

The name *Armeria maritima* subsp. *halleri* was given by Werner Roth in *Feddes Repert. Spec. Nov. Regni Veg.* 67: 9. (1963), which descends the rank of subspecies, rank recognized in the most papers consulted by us.

Other synonyms for *Armeria maritima* subsp. *halleri* (Wallr.) Rothm. are:

- *Armeria bottendorfensis* A.G. Schulz – cf. O. Schwarz in *Mitt. Thuring. Bot. Ver.* n. f. 42: 33 (1936)
- *Armeria elongata* (Hoffm.) W.D.J. Koch var. *serpentini* Gauckler – *Ber. Bayer. Bot. Ges.* 30: 20 (1954)

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- *Armeria elongata* subsp. *serpentini* (Gauckler) Holub – *Novitates Bot*., *Praha* /1960/: 3 (1960)
- *Armeria halleri* sensu Cadevall, Fl. Catalunya 4: 450??? (1932)
- *Armeria maritima* subsp. *calaminaria* (F. Petri) W. Ernst, nom. inval. (sine basion.) – Abh. Landesmus. Naturk. Münster Westfalen, 27 (1965)
- *Armeria maritima* subsp. *bottendorfensis* (A.A.H. Schulz) Rothm.
- *Armeria maritima* subsp. *hornburgensis* (A. A. H. Schulz) Rothm.
- *Armeria maritima* L. subsp. *serpentini* (Gauckler) Rothm. – *Feddes Repert*. *Sp*. *Nov*. *Regni Veg*. 68: 9. (1963)
- *Armeria mulleri* A. Huet - *Ann*. *Sci*. *Nat*., *Bot*. III, 19: 255 (1853)
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European endemit, the plant is indicated in Flora Europaea Search Results as *Armeria maritima* (Mill.) Willd. subsp. *halleri* (Wallr.) Rothm., from: Ga, Ge, Ho, Hs, Po; în Euro+Med Plantbase as *Armeria alpina* subsp. *halleri* (Wallr.) Nyman, from: Cs, Ga(F), Ge, Ho, Hs(S), Po (Map. 1).

According WISSKIRCHEN & HAEUPLER (1998), *A*. *maritima* subsp. *halleri* s. l. has five endemic taxa:

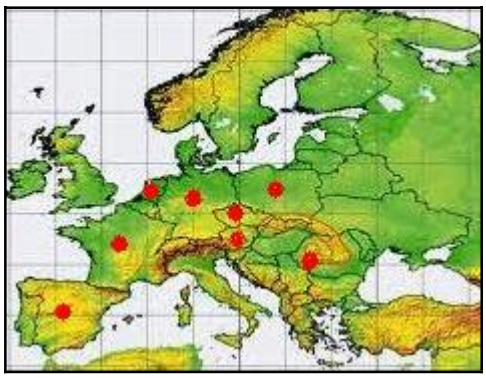
1) subsp. *halleri* (Wall.) Löve & Löve. Basin of Mansfeld (East Germany) Basin of Olkusz and Boleslaw (Poland).

2) subsp. *bottendorfensis* (A. Schulz) Rothm. Metalliferous hills at Bottendorf near Artern (East Germany).

3) subsp. *hornburgensis* (A. Schulz) Rothm. Copper wastes at Hornburg near Eisleben (East Germany).

4) subsp. *calaminaria* (Petri) W. Ernst 1965. Zinc-lead mine wastes near Aachen (West Germany) and Eupen (Belgium).

5) subsp. *eifeliaca* (Petri) Lefèbvre 1972: comb. nov. Near Mechernich in Eifel (West Germany).



Map 1. Distribution of *Armeria maritina* subsp. *halleri* in Europa

According LEFÉBVRE (1974), the five taxa are often put together in a single "metallophyte" taxon. Molecular genetic studies also demonstrated the emergence of multiple heavy metal clans of *Armeria maritima* subsp. *elongata* and their allocation in favor of as varieties or accessions to this subspecies. The following classification of metallophytes, adapted from LAMBINON & AUQUIER (1964), *A*. *maritima* subsp. *halleri* is a facultative metallophytes. They also occur in distinct non-metal enriched phytogeographical areas. The highly specialized ecotype, subspecies or genotype is dependant on the occurrence of specific metals in the soil (BAUMBACH & HELLWIG 2007; BAUMBACH & SCHUBERT 2008).

Species was discovered in Romania by Grecescu, above 1898, in his journey to Godeanu Mountains by Țesna Valley, and since then has not been reported.

We are in a very advanced stage with comparative study of the flora from areas with serpentine from Geopark Plateau Mehedinți, including those in the Ciolanu Mountains is the largest (Ciolanului Mare), where scale is the most (between 810 - 1210 m). This area (least studied of botanical point of view) host and other plants particularly important in scientific and phytogeographic terms: *Plantago serpentina*, *Asplenium cuneifolium* (*A. serpentini*), *Notholaena marantae*, *Gentianella germanica*, etc.

## MATERIAL AND METHODS

This study is based on living plants mainly from Geopark Plateau Mehedinți and herbarium specimens. During botanical research carried out in the Geopark Plateau Mehedinți, we found *Armeria maritima* L. subsp. *halleri* (*Plumbaginaceae*) on serpentine well represented in the Geopark. Distributions covering the focus region were drawn in a GIS, based on the revised herbarium specimens and geographical coordinates of their localities. Localities and habitat notes, especially type of geological substratum, were summarized and habitat preferences of species. Plant material was photographed, collected and herborized. Herbarium acronyms follow HOLMGREN et al. 1990.

## RESULTS AND DISCUSSIONS

DIMITRIE GRECESCU identified our plant under the name of *Armeria elongata*, the name by appearing in his epitome (GRECESCU 1898): „*A. elongata* Boiss. in DC. prodr. 12 p. 681 (1848) e sect. „Pleurotrichae" et spiculae intra involucrum sessiles, folia inter se subconformia. *Statice elongata* Hoffmm. deusch. fl. vol. 1 p. 150 (1791); Koch syn.p. (682. *Statice armeria* L. fl. suec. p.99 (1755) ex p.; Sturm deutsch.fl. h. 1. tab. 5. *Armeria vulgaris* Willd. enum. h. berol. p.133 (1809); Rchb. icon. germ. vol. 17 tab. 98 fig. 2. — 11 Iulie, August. Păsciuni, poeni, în regiunea montană inferioară. În Mehedinți pe poena Ciolanului la 890 m, altit." (GRECESCU 1898: 485).

Information was taken and the Romanian Flora (RĂVĂRUȚ 1960) [*Armeria elongata* (Hoffm.) K. Koch, syn.: *Statice elongata* Hoffm.; *S. armeria* β. *elongata* DC. in Lam. et DC.; *Armeria vulgaris* Willd. β. *elongata* Mert. et Koch in Röhl.]. CIOCÂRLAN (2009) indicates the species *Armeria elongata* (Hoffm.) K. Koch as subspontaneous in Transylvania.

According BAUMBACH & HELLWIG (2007), metallophyte microendemics *A. maritima* -„*hornburgensis*", „*bottendorfensis*", „*eifeliaca*", „*calaminaria*"-should not be given formal rank as varieties or subspecies within *A. maritima*. Also their treatment as varieties of an *A. maritima* subsp. *halleri* s. l. is doubtful because subsp. *halleri* can not be consistently characterized throughout its geographical range and may be an artefact itself. All investigated metallophyte forms show close relations to *A. maritima* subsp. *elongata* (leaves – 15-150 × 1,1-2,5 mm, linear, usually ciliate. Scapes mostly more than 25 cm, glabrous. Capitula (15-) 18-25 mm wide; outer involucral bracts up to 25 mm, as long as or longer than the inner, long-acuminate-subulate. Flower pedicels same length as calyx-tube. Corolla pale-pink. The same authors consider that a taxonomical recognition should be considered necessary it is advisable to treat the microendemics as varieties of that subspecies.

The differences between *A. maritima* subsp. *halleri* and the rest of subspecies group *A. maritima* s. l. are shown in the following key:

1a. Capitula no more than 15(-17) mm wide.....*A. maritima* subsp. *halleri* and subsp. *barcensis*

1b. Capitula more than 15 wide …….....all subsp. from *A. maritima* s. l.

**Description** by Flora Iberica and Flora Europaea

Caespitose perennial herb. Leaves 20-50(60) × 0,5-1,5(2) mm, erect or erect-patent, linear, subobtuse, sometimes mucronulate, with 1-1 + 2 nerves, flat, glabrous, with scarious margins or rarely ciliate. Scapes (7)8-15(23-30) cm, glabrous. Seath 10-15 mm. Capitula 15-18 mm wide. Involucral bracts 10-14, most times terns, amber or yellowish colored, often with purple spots; outer bracts slightly coarse, ovat-lanceolate, frequently acuminate, slightly shorter and wider as the median; median bracts ovate or broadly ovate, mucronulate; internal bracts ovate-oblong. Spikeletes sesile or subsesile; spikelete bracts a wider that internal involucral bracts; flowers - hermaphrodite. Calyx (5)5,5-6,5 mm, holopleurotic, with secondary ribs less obvious and slightly hairy; flower pedicels 0,2-0,4 mm, less than ¼ from the calyx tube length; calyx-lobes 1,3-1,7 mm, triangular-ovate, aristate, generally with length less than ¼ from calyx. Corolla deep pink to purple. Fruit capsule. Pollination entomogame. 2n = 18.

**Ecology**

According Flora Europaea the species grows on pastures dry and stony soils rich in heavy metals or over serpentine. The subspecies *A. maritima* subsp. *halleri* grows only on heavy metal heaps in European plains, and can be differentiated morphologically only with difficulty from the subspecies maritime of the coastal salt marshes or the alpine subsp. *alpina* (leaves - width less than 2 mm; seath less than ½ from involucre's diameter = (15-) 18-30 mm; involucral bracts more terns: external ovate, obtuse, clearly shorter than the median. Flower pedicels long as ½ from calyx-tube).

*A. maritima halleri* has special glands, developed from stomata apparently [similar to sp. *A maritima* utilized to remove excess salt (LÜTTGE 1975; ROZEMA et al. 1981).)], which used to transfer the surplus of heavy metals to the outside (SHERAMETI & VARMA 2011).

Of the 18 serpentine appearance of the whole Geopark Mehedinți, investigated so far, *Armeria maritima* subsp. *halleri* was found only where the altitude is over 800 m, in 5 choronyms. The surfaces vary from one to another choronyms. The largest areas are in the Ciolanul Mare Mountain, where scale is the largest (at 810 to 1210 m). The surface in hectares is difficult to establish, because of the bumps and partial coverage of the forest. Vegetation coverage also varies: where the material is friable, coverage can reach up to 100%. In places with tougher rock the vegetation difficult installed and only cliffs crevices, and the number of species that colonize these places is very low. Usually are very interesting species (*Notholaena marantae*, *Asplenium cuneifolium*, *Silene* ?*bupleuroides*, *Selaginella helvetica*, *Galium flavescens*, etc.). Usually, on the surfaces with compact serpentines, the vegetation is not completed, and where it formed ravines, dominant species is *Achnatherum calamagrostis*.

**Coenology**

In the more eastern parts of Central Europe, metallicolous vegetation with *Armeria halleri* species as exclusive character of the alliance *Armerion halleri* Ernst 1965. The *Armerion halleri* includes the associations of the *Armerietum halleri* Libb. 1930, the *Armerietum hornburgensis* Schub. 1974, the *Armerietum bottendorfensis* Schub. 1953, and the *Holco-Cardaminopsietum* halleri Hülb. 1980 (SCHUBERT et al. 2001; DIARCHIES & BECKER 2008).

The following species are the permanent cohabitants, in all points studied in Geopark: *Plantago serpentina*, *Asplenium cuneifolium*, *Dorycnium germanicum*, *D.*

*herbaceum*, *Poa pumila*, *Potentilla arenaria* subsp. *tommasiniana*, *Silene* ?*bupleuroides*, *Achnatherum calamagrostis*, *Notholaena marantae*. *Plantago serpentina* and *A. maritima* subsp. *halleri* sometimes cohabiting or not. I noticed the same situation across species *Notholaena marantae*.

Between the species rare which seem to be serpentine-tolerant mention: *Achillea crithmifolia*, *Aira elegantissima*, *Euphrasia illyrica*, *Allium flavum* subsp. *flavum*, *Asyneuma canescens*, *Aurinia petraea*, *Cleistogenes serotina*, *Bromus riparius* subsp. *riparius*, *Carlina acanthifolia* subsp. *acanthifolia, Cynosurus echinatus*, *Orchis morio* subsp. *picta*, *Orchis tridentata*, *Ornithogalum orthophyllum* subsp. *kochii*, *Petrorhagia saxifraga*, *Poa pumila*, *Potentilla arenaria* subsp. *tommasiniana*, *Quercus dalechampii*, *Rorippa pyrenaica*, *Scleranthus perennis* subsp. *perennis*, *Seseli peucedanoides*, *Silene armeria*, *Spergularia rubra*, *Thlaspi dacicum* subsp. *banaticum*, *Thymus longicaulis*, *Thymus pulegioides* s. l., *Tragopogon balcanicus*, *Trifolium strictum*, *Viola tricolor* subsp. *tricolor*, etc.

Figure 1. *Armeria maritima* subsp. *halleri*

It seems that the taxonomic information, as morphological and genetic investigations were made in Central and particularly Western Europe, is based exclusively

on plants growing on former mining heavy metals, while we researched plants only on serpentine in situ (on the substrate does not result from the exploitation of heavy metals).

**Chorology**

According Flora Europaea - dispersed in Central and Western Europe, from the Pyrenees and the Netherlands to Poland.

In the area Geopark Plateau Mehedinți:

Poiana Ciolanului, lower mountain region, pastures, meadows, alt. c. 890 m, D. Grecescu [BUC]. *Mehedinți Mountains*, Geopark Plateau Mehedinți, Gornenți NW, Ciolanul Mare Peak, meadow, 44°56′18.45″N, 22°31′05.70″E, alt. c. 1121 m, 23 VIII 2010, G. Negrean (N 15.640) [BUCA; CAREI; CL; HGN]. Gornenți NW, Montes Ciolanul Mare, serpentine, 44°56′07,079″N, 22°30′51,453″E, alt. c. 1020 m, 9 V 2011, Ioana Ciortan & G. Negrean (N 15.837) [CL; BUCA; HGN]. Mehedinți Mountains, Poienile Ciolanul Mic, cacumine ut Ciolanul Mare, serpentine, in herbosis, 44°56′17.188″N, 22°31′19.939″E, alt. c. 1210 m, 14 VII 2011, G. Negrean & Ioana Ciortan. Gornenți N, ad cacumine Ciolanul Mic, in herbosis, 44°56′40.53″N, 22°31′58.13″E, alt. c. 1022 m, 10 VI 2010, G. Negrean (N 15.275) [BUCA]. Montes Ciolanul Mic, serpentine, in herbosis, 44°56′39.697″N, 22°31′58.013″E, alt. c. 1210 m, 14 VII 2011, Ioana Ciortan & G. Negrean. (N 16.143) [BUC; BUCA; CAREI; CL]. Mehedinți Mountains, ad pedem Montes Ciolanul Mare, serpentine, in saxosis, 44°55′57.48″N, 22°30′58.05″E, alt. 977 m, 15 VI 2010, G. Negrean (N 15.445) [CL]. Gornenți WNW, ad pedem Montes Ciolanul Mare, serpentine, in saxosis, 44°55′57.48″N, 22°30′58.05″E, alt. 977 m, 19 VIII 2011, G. Negrean & Ioana Ciortan (N 16.359) [HGN]. Gornenți NW, ad pedem Montes Ciolanul Mare, serpentine, 44°55′48,155″N, 22°30′58,246″E, alt. c. 900 m, 9 V 2011, Ioana Ciortan & G. Negrean (N: 150.832 [BUCA; CL; HGN]. Gornenți WNW, Ciolanul Mare, „La Funduri", „Mlaştina cu *Menyanthes*", 44°55′38.180″N, 22°30′45.054″E, alt. c. 810 m, 19 VIII 2011, G. Negrean & Ioana Ciortan (N 16.359) [HGN]. Pietrele Vinete, serpentine, 44°53′34.770″N, 22°29′46.595″E, alt. 1015 m, 20 VIII 2010, G. Negrean. Podeni WNW, Pietrele Vinete, serpentine, 44°53′34.770″N, 22°29′46.595″E, alt. 1015 m, 17 VIII 2011, Ioana Ciortan & G. Negrean. Mehedinți Mountains, Montes Comorişte, serpentine, in saxosis, 44°52′30.259″N, 22°28′42.297″E, alt. 870 m, 15 VI 2010, G. Negrean (N 15.407) [CL]. Mehedinți Mountains, La Comorişte, serpentine, 44°52′34.375″N, 22°28′38.372″E, alt. c. 900 m, 24 VIII 2010, G. Negrean (N 15.659) [BUCA; CL].

The plant was introduced in the collection from Botanical Garden „Al. Buia" Craiova, Plant Systematic sector, in the summer of 2011. Has adapted very well, blossomed and has produced viable seeds.

## CONCLUSIONS

*Armeria maritima* subsp. *halleri* is specific plant from serpentine, ultrabasic rocks.

According to our information, species was not cited in Romania (RĂVĂRUȚ 1960; OPREA 2005) and perhaps even of the entire Carpathians Chain (MARHOLD & HINDÁK 1998; KIRÁLY 2009; KIRÁLY, VIRÓK & MOLNÁR 2011).

We researched plants only on serpentine in situ (on the substrate does not result from the exploitation of heavy metals).

The altitudes appearance with serpentines (where we found the *Armeria*), varies at 810 to 1210 m.

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# *CONYZA BONARIENSIS*, A NEW PLANT WITH INVASIVE CHARACTER IN ROMANIAN FLORA

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***Keywords***: *alien plants*, *Romanian Flora*, *Conyza bonariensis*, *invasive species*

## ABSTRACT

*A new alien plant species – Conyza bonariensis – is reported from Mehedinți County, Romania. We provide information about its morphological features, distribution, biology, ecology and invasiveness.*

## INTRODUCTION

The genus *Conyza* Less. (*Asteraceae*) comprises more than 50 species, chiefly of warmer regions (MUNZ 1959).

*Conyza bonariensis* (L.) Cronquist is a thermo-cosmopolitan species originating from tropical America (MARTINČIČ et al. 1999).

From Germplasm Resources Information Network (GRIN) (as *Erigeron bonariensis* L.), the plant is native from Southern America and naturalized in: Africa, Asia-temperate, Asia-tropical, Australasia, Northern America, Pacific, Southern America, Europe: Northern Europe, Middle Europe (Belgium; Germany; Netherlands; Switzerland), Southeastern Europe [Albania; Bulgaria; Croatia; Greece (incl. Crete); Italy (incl. Sardinia, Sicily); Macedonia; Montenegro; Slovenia], Southwestern Europe [France (incl. Corsica); Portugal; Spain (incl. Balearic)].

In Europe it was introduced much later than *Conyza canadensis*, and has been naturalized almost throughout the Mediterranean region and the south-western parts of Europe (CRONQUIST 1976). Findings out of this range, such as the case of an occurrence of *Conyza bonariensis* in the British Isles (STACE 1997) and the Czech Republic (PYŠEK et al. 2002; ŠIDA 2002; ŠIDA 2003) are very rare, is reported as new alien species to the Czech Republic - was collected in 1964 and 1965 in two localities in N Bohemia. In both cases, the plants were introduced with cotton and occurred in areas of textile factories. It is established in the neighboring countries of Croatia: in Italy (PIGNATTI 1982), Slovenia (MARTINČIČ et al. 1999) and Montenegro (ROHLENA 1942). The first reliable data for Croatia are given by ROHLENA (1923), as *Erigeron linifolius* Willd., for the area of Dubrovnik, and somewhat later by BOLZON (1925), as *Erigeron crispus* Pourr., for Bay of Kvarner (Mali Lošinj and Krk). Since then it has been regularly noted as a naturalised

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species in the floras of the territory of today's Croatia (HAYEK 1931; DOMAC 1950, 1967, 1994; NIKOLIĆ 2000). In Bulgaria: according DIMITROV & TRIFONOV (2006) species is indicated at Black Sea Coast (*Southern*): Bourgas (DIMITROV & TRIFONOV, 2006) as *Erigeron bonariensis* L., but the indication is incorrect and should be referred to *Erigeron sumatrensis* Retz. In 2009 VLADIMIROV, PETROVA & YANKOV reported from Black Sea Coast (*Northern*): along the railroad tracks at the railway station in the city of Varna, a single plant. In Greece - Chalki, Rodos and reported from nearly all E Aegean islands; Samothraki: SW of Xiropotamos, Kamariotissa (BIEL & TAN 2007, 2009).

Closer to our geographical area, plant occurs in a catalog of seeds for exchange from Botanical Garden from Budapest (RICHTER 1872).

The species is cultivated ground and waste places; naturalized in the Mediterranean region and S. W. Europe.

## MATERIAL AND METHODS

Material was collected to Bahna Valley and Drobeta Turnu-Severin railway station. The plant is deposited in the Herbarium of the University of Cluj (CL) and was compared with material collected by us in Southern France: ***Conyza bonariensis*** (L.) Cronq. /AST/ - **Ga**: St. Raphael N, Esterel, 9 X 1996 [BUCM] (TĂNASE & NEGREAN 1997: 64♪). Fungi: *Podosphaera fusca* (Fr.) U. Braun & S. Takam. (*Oidium erysiphoides* Fr.▼; *Sphaerotheca erigerontis-canadensis* (Lév.) L. Junell; *Sphaerotheca fusca* (Fr.) Blumer♪).

## RESULTS AND DISCUSSIONS

**Nomenclature**. The accepted name for this taxon is *Conyza bonariensis* (L.) Cronquist, Bull. Torr. Bot. Cl. 70: 632 (1943).

The heterotypic and homotypic synonyms are:

≡ *Erigeron bonariensis* L., Sp. Pl. 2: 863 (1753);

= *E. crispus* Pourr., Mem. Acad. Toul. 3: 318 (1788);

= *E. linifolius* Willd., Sp. Pl. 3: 1955 (1803);

= *Conyza ambigua* DC., Fl. Fr. 6: 468 (1815);

≡ *Erigeron ambiguus* (DC.) Sch. Bip. in Webb & Berthel., Phyt. Canar. 2: 208 (1844).

≡ *Conyzella linifolia* (Willd.) Greene, Fl. Francisc. 4: 386 (1897);

≡ *Leptilon bonariense* (L.) Small, Fl. S. E. U. S.: 1231 (1903);

≡ *Leptilon linifolium* (Willd.) Small, Fl. S. E. U. S.: 1231 (1903).

**Etimology** (according Wild Plants of Malta):

- ***Conyza*** – *Conyza* – derived from Greek word „Konops" which means flea, and used Pliny as a name for some kind of a fleabane. **Fleabane** is a common name for some flowering plants in the family *Asteraceae*, subfamily *Asteroideae* (*Conyza*; *Erigeron*; *Inula*; *Pluchea*; *Pulicaria*), and one genus from subfam. *Cichorioideae*: *Vernonia*.

- ***bonariensis*** – referring to the fact that the plant may have originated from Buenos Aires in Argentina, South America.

The identification key includes *Conyza canadensis*, *Conyza bonariensis* and *Conyza sumatrensis*, species may be confused (ŠIDA 2003, WURZELL 1988).

1a Ligulate florets present, white, of 0.5-1 mm; involucral bracts glabrous or hairy scattered…….....

***Conyza canadensis*** (L.) Cronquist - Annual plants, up to 10-150 cm, patent hirsute. Leaves numerous, narrow, the lower up to 10 x 1 cm, oblanceolate, petiolate, often deciduous, the others linear, at least the upper sessile. Capitula less than 1 cm wide, generally numerous, in

a long, paniculate inflorescences with a single axis. Involucres 3-4 mm, equaling or slightly exceeding the style and pappus. 2n= 18.

1b Ligulate florets absent or very short, not more than 0.5 mm, oft en with reddish apex; involucral bracts usually hirsute……2

2a Inflorescence rhombic; ligules very short, up to 0.5 mm, head 3-7 mm……

***Conyza sumatrensis*** (Retz.) E. Walker - Annual plants, up to 232 cm. Leaves are numerous, simple, alternate, the lower elliptic-lanceolate to oblong-ovate, petiolate, remotely dentate (with 3-6 teeth on each side), the middle linear-lanceolate to linear, 4-10 x 0.6-1(1.2) cm, ± entire, the upper shorter and narrower, sessile. Inflorescence is rhombic in outline, 30-50 cm long, with many heads (up to 1200 to an individual of about 200 cm height); involucrum 4-6 x (4)5-7 mm, bracts grayish-green, linear lanceolate, acuminate, densely hairy; female florets 110-200, with very short whitish ligules; hermaphrodite flowers *ca*. 15, with yellow corolla; achenes 1-1.5 mm long, with 3-5 mm long pappus of pale brown hairs (ANASTASIU and DANIYAR, 2012).

2b Inflorescence pyramidal, with elongate branches overlapping the main axis; ligules absent; head 7-10 mm……

***Conyza bonariensis*** (L.). Cronquist – Annual plants. Erect **stems** (can be 20-75 (200) cm in height) covered with two kind of hairs, short ones, strongly appressed to the stem, arranged in a dense indumentum and long ones, patent, scattered. **Branching** extensively at the base, decreasing upwards. **Leaves** linear or linear lanceolate (narrow lanceolate), entire, 0.1–0.5 cm wide and 5–8 cm long, coarsely toothed and covered in fine hairs. Upper leaves are smaller and linear. **Inflorescence** cylindrical, usually in paniculiform to racemiform, rarely corymbiform arrays, composed of 15–40 capitula per branch. **Capitulas** 5–7 (10) mm in diameter, without ligulate flowers. Involucral bracts 3–4 (5) mm long, (leaf-like structures surrounding the flower head) have a brownish inner surface and the hairs (pappus) of the seed are cream the outer ones covered **with appressed hairs**, the inner ones hairy only along the midrib; Flowers occur at the ends of the branches. **Disc florets** 8–12 or more. **Pistillate florets** 60–150 or more, light-yellow; corollas ± equaling or surpassing styles, laminae 0 or to 0.3 mm. Surrounding each flower are involucral (bell-shaped leaf-like) bracts 3-5mm in length, the inside of each bract is white sometimes tinged purple or red. Achenes linear-lanceoloid, compressed, c. 1.5 mm, sparsely strigullose [or glabrous] straw colored, covered in hairs with 16-20 noticeably longer at the top that are white or pink, sordid, or tawny bristles 3–4 mm or more.

**Presence in Romania**

In the Romanian botanical literature, Simonkai (1886) mentions, with the doubtful presence, a plant under the name *Erigeron crispus* Pourr. [syn.: *Conyza ambigua* DC., *Erigeron transsilvanicus* Schur. (leg. Schur 1845, Dobra and Zam, county Hunedoara)], plant with intermediate characters between *Conyza canadensis* and *Erigeron acris* (incl. *E. podolicus*). SIMONKAI considers the fictional presence of this species in the Transylvania.

Figure 1. *Conyza bonariensis*

Figure 2. *Conyza canadensis* (left) and *Conyza bonariensis* (right)

SÂRBU & OPREA (2011) considered the species *Conyza bonariensis* one and the same species indicated by SIMONKAI (1886) -*Erigeron transsilvanicus*- and hypothesize that it may be a natural hybrid between *Conyza canadensis* and *Erigeron acris* species, known in the literature as the × *Conyzigeron huelsenii* (Valke) Ruaschert.

However, according (CRONQUIST 1976), *Conyza bonariensis* was introduced in Europe than later as *Conyza canadensis*, so before 1866 the plant does not exist in Europe. This means that the *Conyza bonariensis* species not the same plant with hybrid ×

*Conyzigeron huelsenii* (Valke) Ruaschert, as involving SÂRBU & OPREA (2011). Moreover, to us the plant has a strong thermophilic (Railway Station Drobeta, Bahna Valley).

**Chorology**:

a. distr. Mehedinți: Cireşul SW, Bahna Valley, in herbosis, 44º48′43,75″N, 22º31′15,48″E, alt. c. 242 m, 27 VIII 2010, leg. G. Negrean (N: 15.461) [CL].

b. distr. Mehedinți: Railway Station Drobeta Turnu-Severin, ad viam ferream, 44º37′17,57″N, 22º38′12,55″E, alt. c. 49 m, 24 VIII 2011, leg. G. Negrean & Ioana Ciortan (N: 15.462) [CL]. 8 VIII 2012, leg. Ioana Ciortan & G. Negrean [GN: 18.702], together with: *Acer negundo*, *Ailanthus altissima*, *Amaranhus deflexus*, *Bassia scoparia*, *Centaurea diffusa*, *Cephalaria transylvanica*, *Convolvulus arvensis*, *Conyza canadensis*, *Dasypyrum villosum*, *Erigeron annuus* subsp. *annuus*, *Gleditsia triacanthos*, *Juglans regia*, *Panicum dichotomiflorum*, *Petrorhagia saxifraga*, *Portulaca oleracea* subsp. *oleracea*, *Tribulus terrestris*, *Sorghum halepense*, etc.

## CONCLUSIONS

1. The paper indicates the certain existence of the species *Conyza bonariensis* as an alien plant in Romania, from Mehedinți County.

2. *Conyza bonariensis* was recently indicated from Romania, as much from the confusion in the ancient literature (commented) and as the fact that one of the authors of the present paper (NEGREAN) communicated to Mr. Adrian OPREA that he found the plant in Mehedinți County. The publication of the species from the two authors (SÂRBU & OPREA 2011) contravenes the professional ethics, because the above authors did not have the permission of Mr. NEGREAN for the publication.

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# THE OCCURENCE AND DISTRIBUTION OF SEROTONIN IMMUNOREACTIVITY IN THE PANCREAS OF SEVERAL AMPHIBIAN SPECIES

Luminiţa Mariana Olaru[1]

***Key words***: serotonin, pancreas, immunoreactivity, amphibians.

## ABSTRACT

*5 - Hydrohitryptamine appears widespread in GEP (gastro-entero-pancreatic) neuroendocrine system of the poikiloterm mammals and vertebrate. Both in the pancreas and in the gastrointestinal tract of some amphibians (Triturus vulgaris, Rana esculenta, Xenopus laevis), it has been observed the coexistence of nervous elements and endocrine immunoreactive for 5-HT. Primary antiserum used, regardless of origin, and immunohistochemical procedure applied, have allowed the sporadic identification of some unique endocrine, immunopositive elements for 5-HT in the pancreas of some amphibians. Also, the results described in this paper are covered by evidence showing good preservation of the molecule of serotonin during development.*

## INTRODUCTION

Serotonin or 5-hydroxytryptamine (5-HT) produced neuroendocrine, having vasoconstriction effects in vertebrates, together with its precursor 5-HTP and catecholamines (noradrenaline, adrenaline and dopamine), form the family of biogenic monoamine, widespread in the tissues and neural and endocrine organs of vertebrates. 5-HT molecule seems to be well conserved during the vertebrate evolution, its presence in enteric neurons being suggested along with the emergence of ancestral chordates (Goodrich et al.,1980).

Serotonin is widespread in neuroendocrine system GEP (gastro-entero-pancreatic) of the poikiloterme mammals and vertebrates, where it fulfills complex functions related to endocrine and exocrine secretion of the pancreas (Masuda et al., 1997; Adeghate et al., 1999) as well as of endocrine secretion and gastrointestinal motility tract (Tzanewa, 2003, Crowell et al., 2004). Finally, it is necessary to mention the alternative of the coexistence of nervous and endocrine immunoreactive elements for 5-HT both in the pancreas (Trandaburu, 1972b; Faraldi et al., 1993) and in the gastrointestinal tract (Valverde et al., 1993; Maake et al., 1999, 2001) of some amphibians.

The above results concerning the presence, nature, ultrastructural characteristics and 5-HT features distribution in the GEP organs, somewhat contradictory, even in a single

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amphibian species (Valverde et al.,1993; Bodegas et al., 1997; Kostiukevich, 2003), led me to investigate the pancreas and intestine of urodeles and anurans.

## MATERIALS AND METHODS

Animals

The research was conducted on a number of three amphibians species that belong to some systemic groups, existing in our country's fauna or purchased from commercial sources (Xenopus laevis).

The immunohistochemical and electromicroscopic investigations were conducted on the following species of amphibians:

Urodela newts Triturus vulgaris (2 specimens)
Anura frogs Rana esculenta (2 specimens)
Xenopus laevis (3 specimens).

Adult specimens of amphibians, of both sexes, captured in spring - summer (March to May) in the surroundings of Craiova city, were kept before sacrifice in tanks with fresh water for 2-4 days. Animals weren't fed during their period of captivity.

Tissue preparation

The animals were sacrificed under chloroform anesthesia and the target organ (pancreas) was quickly excised and divided into fragments of different length, depending on the species (0,5-1,5cm) in order to compare the results obtained from several species.

The whole pancreas or fragments of it ($\leq$ 1 $cm^3$) were fixed in Bouin's fluid (Romeis, 1968) through immersion at the lab's temperature for different periods of time, according to the methods used to color sections (immunocolors 16-36 hours; colors for pointing out the tissue structures – 2 - 4 days).

Furthermore, the samples have been cleaned, at least in 24 hours in streams or in several series of the tampon solutions, where the fixing solutions have been prepared. Cleaning was followed by dehydration of the samples in graded ethanol series and of their purification in three toluene series. At the end, tissue samples were included into paraffin.

Paraffin segments have been sectioned by a sledge microtome (E. Leitz, Germany). The sections obtained of 5-7 μm-thickness, have been placed on microscopic slides that were first treated with albumin for histology observations or other water solutions of 0,2% gelatin or of 1% poly-L-lysine (Sigma, USA) for the immunohistochemical observations.

Primary antiserum

The primary antiserum – polyclonal rabbit anti-synthetic 5-HT – has been purchased from Incstar, Stillwater, Minn., USA and Biotrend, Cologne, Germany (Larsson, 1981).

Immunohistochemical protocol

For the evidence of 5-HT PAP methods were used (peroxidase anti-peroxidase) and ABC (streptavidin / avidin-biotin-peroxidase) in initial forms (PAP - Sternberger, 1974; ABC - Hsu et al., 1981) or modified under the terms of the laboratory and research objectives). Primary antibody dilutions applied in the ABC method was 1:10000 and in the PAP method - 1:80000. During investigations, I used as a secondary layer of the reaction:

a. in PAP method:

- goat anti-rabbit lgG, delivered by the companies Behring (Germany), Dakko (Denmark), Biotrend (Germany) and UCB Bioproducts (Belgium) or
- the complex formed by goat anti-rabbit lgG and peroxidase entirely belonging to the molecule of the whole mule antibody (ECL, England).

b. in the ABC method – peroxidase:

- goat anti-rabbit lgG, to which biotin was added, a product of Sigma, Jackson Immuno Research and Qualex, companies, all of them from the USA.

The third layer of the reaction was, in the PAP method, the complex of the same name obtained from the companies like Dakko (Denmark), Sigma (USA) and Biotrend (Germany), and in the case of the ABC method, streptavidin-biotin-peroxidase complex, purchased from Qualex and Jackson Immuno Research (USA) and avidin-biotin-peroxidase (Sigma, USA).

The antigen-antibody binding sites were visualised by a 5 min. – immersion of the section in a solution of 0,7 mM 3,3' – diaminobenzidine tetrahydrachloride (Serva Feinbiochem, GmbH., Germany) preparated in 0,05 M Tris-HCl buffer (pH 7,6), to which 0,02% hydrogen peroxide was added just before use. 0,01 M phosphate buffer saline (PBS) (pH 7,4) was used as diluent for every step of the procedure and as rinsing solution between each step. The sections were finally dehydrated in ethanol, cleared with xylene, mounted in Entellan (E. Merck, Germany) and examined in a Zeiss (Oberkochen, Germany) Photomicroscope II.

Specificity controls

The control of the specificity of immunohistochemical methods and the primary antibody has been done according to the recommendations in the literature (Grube et al., 1986; Cetin et al., 1989, 1993; Trandaburu et Nürnberger, 1995).

a. Method specificity's control

- the use of some gradual ascending dilutions of primary antibodies;

- the replacement of the incubations in the first (primary antibody), the second (IgG or IgG biotinilat) or the third (the PAP complex, avidin-peroxidase complex) layer of the immunohistochemical method with incubations in PBS;

- poly-L-lysine (2mg/ml) addition to primary antibody;

- molar growth (0.5 M) of PBS solution used for cleanings within the immunohistochemical procedure.

b. Primary antibody specificity's control

- preadsorption of primary antiserum with variable quantities of homologous (corresponding) or heterologous antigens, but structurally related. The appliance of the 24 preadsorpted antiserum at a temperature of 4ºC,with its corresponding antigens even of the dilution of 8μg/ml antibody had the direct result the lack of the immunocolors while the use of preadsorpted antiserum with antigens structurally related (till the concentration of 128μg/ml antibody) did not affect the specific immunocolor.

Primary antibody specificity, regardless of procurement source was controlled by preadsorption with its homologous antigen (5-HT synthetic - ICN Pharmaceuticals, USA) or its precursor (5-HTP synthetic-ICN Pharmaceuticals, USA).

For electron microscopy observations, fragments of the pancreas belonging to the specimens of Triturus vulgaris and two species of anuria (Rana esculenta and Xenopus laevis) that were studied were fixed in buffered glutaraldehyde, processed in accordance with the procedures described and included in the hydrophobic medium (812 Epone or Durcupan ACM).

Conventional ultrastructural researches

Tissues preparation included the following stages: sampling small tissue fragments (1-2 mm3) from different regions of the Triturus vulgaris and Rana esculenta species pancreas; immersion fixing the tissue fragments (2-4 h) in a fixing buffer solution

of 2,5% glutaraldehyde (GDA); postfixing the tissue fragments in a buffer solution of 1,3% OsO4 (osmium tetraoxide); cold dehydration of the samples in increasing concentrations of ethanol solutions; impregnation of the tissue fragments with expoxidic resins for including in Durcupan ACM (Fluka, Switzerland); division of the tissue fragments included in expoxidic resins with the Tesla BS – 490 A ultramicrotome; successive staining of ultrafine sections collected grids, with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963); examination and photographing the preparations with the electronic microscope Tesla BS-500.

## RESULTS AND DISCUSSION

Primary antisera used, regardless of origin, and immunohistochemical applied procedure, have allowed the sporadic identification into the amphibians' pancreas of some individual endocrine elements, imunopozitive for 5-HT (Fig. 1a-c).

In comparison to newts' pancreas, in both anurans species, the incidence of 5-HT cells was much lower; in a section through the organ only 1-3 imunocolorate elements being identified. In all amphibians, neither topographic distribution nor serotonin cells' form could be correlated with their intra-or extra island membership. Finally, it is appropriate to mention the absence of neural immunoreactive structures (nerve fibers and / or neurons) in the organ of investigated amphibians.

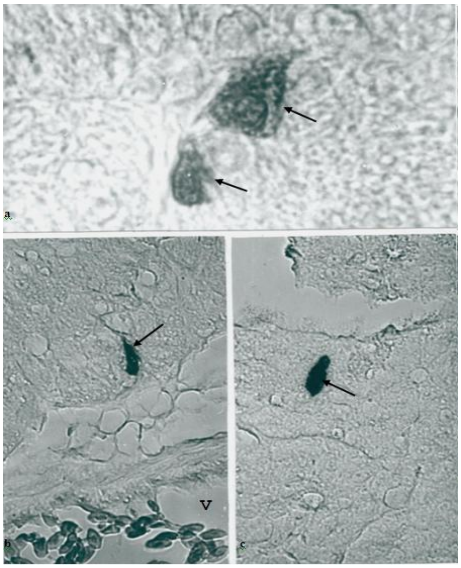


Fig. 1a-c. Serotonin cells (arrows) in the pancreas of Triturus vulgaris (a), Xenopus laevis (b) and Rana esculenta (c). V = blood vessel with red blood cells. a. - ABC method - peroxidase, primary antibody dilution of 1:10000;
b, c. - PAP method, primary antibody dilution of 1:8000. a × 1000; b, c × 160.

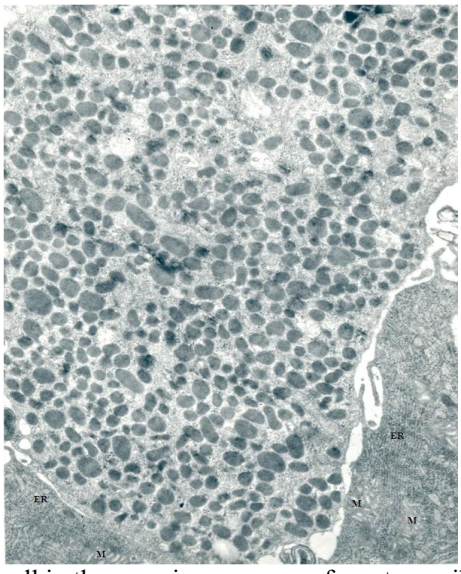


Fig. 2. Single cell in the exocrine pancreas of newt, possibly producing of 5-HT. Different sizes and heterogeneous form of secretion granules are noted. ER = Ergastoplasma; M = mitochondria. × 23 600.

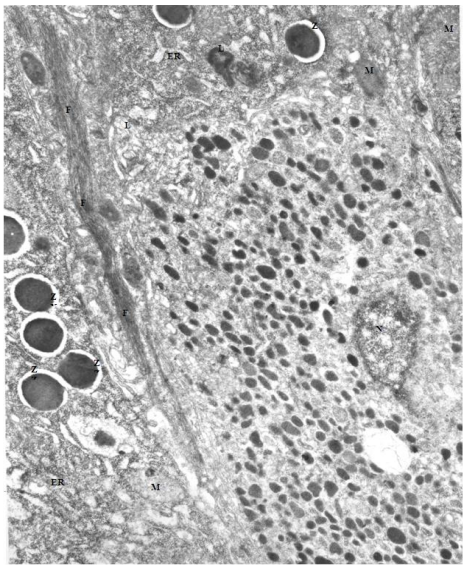


Fig. 3. Possible serotonin-producing cell identified in the pancreas of Rana esculenta frog. Most of the secretion granules have an elongated shape and are widely osmiofile. N = nucleus; Z = zimogen granules; ER = ergastoplasma; M = mitochondria; F = connective tissue fibers, L=lysosomal. × 13800.

5-HT ultrastructural appearance of pancreatic chromaffin cells examined in Triturus vulgaris and Rana esculenta, is characterized, like those of mammals and humans (Solcia et

al., 1969), by the polymorphism of secretion granules with higher dimensions (306 ± 52 nm) towards the granules of island hormones (Fig. 2, 3). In Rana esculenta frog's pancreas, most of the secretion granules of the serotonin-producing cells have an elongated shape and are widely osmiofile, while in the exocrine pancreas of Triturus vulgaris, various size and heterogeneous shape of the secretion granules in a singular cell, possible productive of 5-HT is seen. The observations on the nature of immunoreactive structures quartered in the pancreas, generally confirm the previous results (L'Hermite et al., 1988; Bodegas et al., 1997; Ku et al., 2000, 2003; Kostiukevich, 2003) regarding the predominant location, if not exclusive, of serotonin in endocrine cells of the GEP organs of amphibians. In the above context, the investigations conducted under optimal methodology, have demonstrated that in the pancreas of Triturus vulgaris, Rana esculenta and Xenopus laevis species the presence of serotonin only in a subpopulation of endocrine cells very limited numerically.

As for the fundamental problem in cell biology of the phylogenetic conservation of the molecular structures of the various products with biological activity quartered in GEP organs and of enzyme equipment related to their production (Bloom and Polak, 1981), research results contained in this paper represent a minor contribution, but original in favor to the concept of their proper conservation during evolution. The fact that, in comparison to the amphibians, species and taxa situated on higher stages of evolution (some reptiles, birds and even mammals) do not express 5-HT or express it only sporadically and neuropeptides identified by us, can be attributed to some "molecular accidents of the evolution" dictated by adaptive needs or of technical imperfections in their detection.

I therefore sought that the address of molecular diversity of the neuropeptidergic compounds of GEP organs would be useful, in order to briefly refer to the multiple possibilities offered by the enterochromafine, pancreatic and enteric cells, of storage of the neuropeptides, glycoproteins and certain islander hormones. In this respect, I mention serotonin's, a chromogranin (CGA)'s co-storage and of some biologically active fragments of secretogranin II (SgII SgII C23-3 C26-3) in the intestine of some anuria and reptiles (Trandaburu and Syed Ali, 1998) or of islander amyloid ( IAPP) and of pancreatic and intestinal serotonin in rats and humans (D'Este et al., 1995).

Given on the one hand, the great incidence of 5-HT enterochromafine elements in some animal species and their multiple availabilities genetically imprinted, of biosynthesis and co-storage and on the other hand the extraordinary diversity of the products with biological activity quartered at enteric level, I believe that today we are still far to achieve comprehensive knowledge of the biosynthetic and secretion potential of immunoreactive cells for serotonin.

## CONCLUSIONS

Compared with the representatives of other classes of vertebrates, 5-HT immunoreactivity appears sporadically and only endocrinal expressed in the pancreas of the investigated amphibians.

(5-HT) serotonin cells, detected only sporadically in the newts' and frogs' pancreas, were for all the amphibians taken under study the most abundant population of enterochromafine cells. Their distribution has been linked to proved implications of the monoamine in triggering the local responses, which were immune and hypotensive or of those which modulated the speed of the enteric transit.

On the ultrastructure of 5-HT cells, the different sizes and heterogeneous appearance of secretion granules have been attributed to the multiple co-storage

possibilities of some regulating peptides or neuropeptides.

The identifying in the amphibians' pancreas of some neural elements (ganglion pericarions and / or nerve fibers) and immunoreactive endocrine cells for 5-HT not only that extends the lower vertebrates range lower but also of the neuropeptidergic compounds quartered in their GEP system, it also turns to be important for the fact that it represents a cogent proof of the perennial phylogenetic of the molecular structure and of the he functions of this amine.

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# THE REPRODUCTIVE HISTORY AND THE RISK OF THE ENDOMETRIAL CANCER

Diana Olimid[1]

***Keywords*:** *endometrium, cancer, menarche, parity, menopause*

## ABSTRACT

*The aim of this study was to correlate the reproductive history with the risk of endometrial cancer. The study included 795 patients with a histopathological diagnosis of endometrial hyperplasia or carcinoma. The results revelead the presence of the menarche under the age of 12 at 40,06% of the patients with hyperplasia and at 39,47% of those with carcinoma.*

*The age at menopause was over 50 years old at the majority of the patients (51,93% and, repectively 59,42%). I also noticed the presence of a history of irregular menstrual cycles at 37,67% of the patients with hyperplasia and at 43,28% of those with carcinoma.*

## INTRODUCTION

Uterine cancer is the second most common cancer of the female genital tract after ovarian cancer (IRC UK 2008). Most of the estabilished risk factos for the endometrial cancer are the result of the persistent exposure to the oestrogen levels unopposed by progestagens. This process stimulates the cell proliferation and increase the risk of the endometrial cancer (Pike et al. 2004).

Uterine cancer has incidence rates more than four times higher in the developed countries. In North America, Australasia and many European countries the incidence of the cervical cancer has fallen with the introduction of screening and endometrial cancer is now the most commonly diagnosed gynecological cancer. The highest rates of uterine cancer incidence are found in Malta, Czech Republic and Luxembourg, Romania, Greece and Portugal have the lowest rates. (Bray F. et al. 2005).

In this study I try to correlate several reproductive factors respectively the age at menarche, the parity, the character of the menstrual cycles and the age at menopause with the endometrial cancer risk. The results obtained were permanently correlated with those from the literature, regarding the studies made by reputed universities and research centres in Europe and in the USA.

## MATERIAL AND METHODS

The study was done during a period of 5 years and included 795 patients with a histopathological diagnosis of endometrial hyperplasia or carcinoma. The cases were divided in two groups according to the diagnosis and investigated anamnestically regarding

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the reproductive risk factors. I evaluated for each group the age at menarche, the parity, the character of the menstrual cycles and the age at menopause.

## RESULTS AND DISCUSSIONS

The evaluation of the reproductive risk factors for the both groups of patients is represented in the next table (Tabel no. 1).

Tabel 1

The evaluation of the reproductive risk factors

| Risk factor | Endometrial hyperplasia | | *Endometrial cancer* | |
|---|---|---|---|---|
| | No. of cases | Percentage % | No. of cases | *Percentage %* |
| The age at menarche | | | | |
| <12 years old | 250 | 40,06% | 68 | *39,77%* |
| 12-13 years old | 166 | 26,60% | 49 | *28,65%* |
| 13-14 years old | 124 | 19,87% | 33 | *19,30%* |
| > 14 years old | 84 | 13,46% | 21 | *12,28%* |
| The parity | | | | |
| Nulliparous | 223 | 35,74% | 38 | *22,23%* |
| 1 | 184 | 29,49% | 52 | *30,41%* |
| 2 | 141 | 22,60% | 46 | *26,90%* |
| ≥ 3 | 76 | 12,17% | 35 | *20,47%* |
| The age at menopause | | | | |
| < 45 years old | 36 | 23,07% | 19 | *13,77%* |
| 45-49 years old | 39 | 25% | 37 | *26,81%* |
| 50-54 years old | 48 | 30,78% | 56 | *40,58%* |
| ≥ 55 years old | 33 | 21,15% | 26 | *18,84%* |
| *Irregular menstrual cycles* | | | | |
| *Present* | *235* | *37,67%* | *74* | *43,28%* |
| *Absent* | *299* | *62,33%* | *97* | *56,72%* |

The menarche is a terme witch define the apparition of the first menstruation. The age at menarche is usually between 10 and 17 years old. (Vartej P. & Vartej Ioana 2012).

Early menarche is considerated a factor that increase the risk of endometrial cancer for 1,5-2 times. The study I made showed that the age at menarche for the majority of the patients with hyperplasia was under 12 years old (40,06%). Only for 84 patients (13,46%) the age at menarche was over 14 years old. The results were simillary in the case of the patients diagnosed with cancer.

Early age at menarche has been described as a risk for endometrial cancer. During the last 150 years the age at menarche has declined at a rate abouth 2 to 3 months per decade (Wyshak G & Frish R.E. 1982, Bray F et al. 2005).

A study made in the USA on a group of women diagnosed with endometrial cancer showed that the percentage of the cases with early menarche was higher (22,43%) in comparison with a control group (16,95%). Also, the late menarche, over 14 years old was present in a lower percentage than in the control group (22,84% and, respectively 29,16%) (Trendham-Dietz A. et al. 2006).

According to the American Cancer Society at 51,62% of patients diagnosed with endometrial cancer the first menstruation occured under the age of 12, at 41,43% of them between 13-14 years old and only at 6,95% of them over the age of 14 (Lacey J. V. Jr. et al. 2007).

Nulliparity is a factor that increase the endometrial risk cancer for 3 times (Persson I. & Adami H. O. 2002). The evaluation of this factor showed that 223 of patients with hyperplasia (35,74%) were nulliparous, 29,49% had one childbirth and only 12,17% had 3

childbirths. In the group of the patients with cancer the percentage of the nulliparous was lower (22,23%). The explication is probably the fact that many of the patients with hyperplasia were under the age of 40, and they didn't end the reproductive period.

A study made between 1946-2004 on a group of 102 women diagnosed with endometrial cancer and ages between 24 and 45 years old showed that 65% of them were nulliparous (Walsh C. et al. 2005).

Another study published by Oxford University showed that the percentage of the nulliparous with cancer was higher in comparison with the control group (16,62% and respectively, 10,59%). The percentage of the women with 3-4 childbirths in their reproductive history was lower in the group studied in comparison with the control group (33,24% and 37,02%) (Trendham-Dietz A. et al. 2006).

According to the American Cancer Society 21,86% of the patients with endometrial cancer were nulliparous, 11,39% had one childbirth, 26,51% had two childbirths and 40,23% over 3 childbirths (Lacey J. V. Jr. et al. 2007).

Pregnancy and parity reduce the risk of cancer by 30% for woman's first birth and by 25% for each succesive birth. The later maternal age at last birth has also been shown to reduce risk. Pregnancy and childbirth are thought to reduce risk through the elimination of the premalignant cells and the break in unopposed oestrogen exposure during pregnancy. Research seems to suggest a greater protective effect of parity on postmenopausal women (Hall G. E. et al. 2002, Bray F. et al 2005).

The change of the reproductive behavior has been reflected on the natality rate among female cohorts born during the 20th century in most European countries. The most substantial decreases occurred following the post-war, so called „baby-boom". The peak of fertility in the 1960s was followed by declines in generations born after 1930s. Nulliparity decreased for cohorts born from 1930-1945 (Bray F. et al 2005).

A study made in Sweden indicated that reproductive patterns may account for abouth half of the cases of endometrial cancer in women diagnosed from 1961 to 2002. The importance of the reproductive behavior on risk has been to decrease at older age (Weiderpass et al. 2000).

The age at menopause is usually between 45 and 55 years old (Vartej P. & Vartej Ioana 2012). The menopause installed naturally over the age of 52 years old increase the endometrial risk by 2,4 times in comparison to the women with the age at menopause under 49 years (Pike M. C. et al. 2004).

The age at menopause for the patients with hyperplasia was in the majority of cases between 50 and 54 years old (30,78%), between 45-49 years for 25% of them and over 55 years old for 21,15% (51,93% over 50 years old). The percentage was higher in the group of women with endometrial cancer, respectively 59,42%.

The results obtained were according to the data from the literature. One study indicated a percentage of 38,74% for patients with the age at menopause between 50-55 years old and a percentage of 21,72% of those with age at menopause over 55 years old. These values were higher then those obtained for the control group (15,65%) (Trendham-Dietz A. et al. 2006).

Conform to the American Cancer Society, the menopause occured at 49,62% of patients between 50-54 years old, at 24,05% of them between 45-49 years old and at 17,47% over the age of 55. The percentage of the cases where the menstrual cycles stoped before the age of 45 was smaller, only 8,86% (Lacey J. V. Jr et al. 2007).

Another reproductive factors associated with increased risk of cancer are irregular menstrual cycles, annovulatory cycles and more days of menstruation (Hall G. E. et al.

2002, Xu W. H. et al. 2004). I observed that the history of irregular menstrual cycles was present at 37,67% of the patients with hyperplasia and at 43,28% of the patients with cancer. A study made on young women with cancer and ages betwwen 24 and 45 years old revealed the presence of the infertility at 18% of them and the irregular menstrual cycles at 37% of them (Walsh C. et al. 2005).

## CONCLUSIONS

Analysing the implications of the reproductive history in the etiopathogenesis of the endometrial proliferative lesions I noticed the the age at menarche was under 12 years old at 40,06% of the patients with hyperplasia and at 39,77% of those with cancer. The menopause was installed after the age of 50 years old at the majority of the patients, respectively at 51,93% of those with hyperplasia and at 59,42% of those with cancer. The history of irregular menstrual cycles was present in antecedents of 37,67% of patients with hyperplasia and at 43,28% of those with cancer.

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# THE ORAL CONTRACEPTIVES AND TABACCO CONSUMPTION – FACTORS WHICH REDUCE THE RISK OF ENDOMETRIAL NEOPLASIA

Diana Olimid[1], Daniel A. O.[2]

***Key words*:** *cancer, endometrium, risk, contraceptives, tabacco*

## ABSTRACT

*The aim of this study was to evaluate the protection factors which reduce the risk of endometrial neoplasia. The study was done on a two groups of women diagnosed with endometrial hyperplasia and carcinoma. I followed two parameters: the use of oral contraceptives and the tabacco consumption.*

*I noticed that 62,82% of patients with hyperplasia and 66,08% of those with carcinoma never used combined oral contraceptives. The majority of the patients with cancer (61,40%) were non-smokers, and only 10,52% of them were continuing smokers.*

## INTRODUCTION

Cancer of the endometrium is the most common gynaecological cancer and ranks one of the most treatble when identified in its earliest stages. It is approximately 40 percent more common in Caucasians as it is in African Americans and other non-Caucasian women. Age is the most important risk factor for death from endometrial cancer. The average women who develops uterin cancer is in her early 60s (American Cancer Society 2008).

There were increasing trends among postmenopausal women in many Northern and Western countries. The endometrial cancer will become a more important public health problem in the future because of the obesity and decreases in fertility (Bray et al. 2005).

The objectiv of this study was to made a analysis regarding the factors which decrease the risk of the endometrial cancer, respectively the consumption of combined oral contraceptives and smoking.

## MATERIAL AND METHODS

In the present study I evaluated two groups of women: the first one was represented by 624 patients with endometrial hyperplasia and the second one was represented by 171 patients diagnosed with cancer.

All the patients were investigated anamnestically regarding the followed factors:

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- the use of the combined oral contraceptives (COC) in their antecedents;
- the duration of the use (under 3 years / for more then 3 years);
- the aspects of the tabacco consumption (non-smokers / smokers in the past / continuing and current smokers).

## RESULTS AND DISCUSSIONS

The evaluation of the oral contraceptives use is showed in the table no. 1.

Table no. 1

The evaluation of the COC use

| Aspects | | Endometrial hyperplasia | | *Endometrial cancer* | |
|---|---|---|---|---|---|
| | | No. of cases | Percentage % | No. of cases | *Percentage %* |
| COC use | No | 392 | 62,82% | 113 | *66,08%* |
| | Yes | 232 | 37,18% | 58 | *33,92%* |
| The term of use | < 3 years | 186 | 80,17% | 41 | *70,69%* |
| | *> 3 years* | *46* | *19,83%* | *17* | *29,31%* |

The comparative study of the two groups showed that 62,82% from the patients with hyperplasia and 66,08% from those with cancer never used combined oral contraceptives. Also, the term of use was under 3 years for the majority of the patients from both lots (80,17% and, respectively 70,69%).

The use of combined oral contraceptives (COC) confers a long-lasting protection from endometrial cancer, particularly among long-terms users. The risk reduction is about 10% for each year of use and has been reported in some studies to persist for as long as 20 years after stopping use (Weiderpass E. et al. 1999, Pike M. C. et al. 2004).

Also, it is considered that the use of COC it is a protection factor from endometrial cancer even after a short period of use, between 1 and 3 years (Dos Santos S. & Swerllow A.J. 1995, Lundberg V. et al. 2004).

An ample study analysed the incidence rates of endometrial cancer in 13 European countries and the changes in several established risk factos over time and has implications for possible primary prevention strategies. The use of hormonal contraceptives may be responsibile for the decreases incidence in several countries. The study observed systematic decreases in incidence of endometrial cancer in women ages 45 to 54 years born in most Northern European countries and in the Czech Republic, France and Italy, with successively declining risk most evident in women born between 1930 and 1945. COC have become increasingly available in Europe from the 1960s onward, and women born after 1925 have had the opportunity to use them. A high exposure to COCs was common among cohorts born after 1950. In England and Wales, the proportion of users of hormonal contraceptives was about 40% for women born in the 1930s compared with ~ 70% in birth cohorts of 1940s to 90% for those born in the 1950s (Bray et al. 2005, ISD Online 2007).

In other European contries, the prevalence of use of COCs has varied, being quite high in Northern and Western Europe, to less common in Southern and Eastern Europe before the 1980s. Increasing incidence rates of endometrial cancer in Eastern Europe (Slovakia, Czech Republic, Slovenia) and in Southern Europe (Spain) have been observed. An explanation for this fact may be that women in these regions have failed to benefit from

the protective effects of COC due to their unavailability. Declines in incidence have been reported in women born after 1920 in Sweden, England, Wales, Germany and Switzerland (this fact due to the increasing prevalence of contraceptives use) (Bray et al. 2005).

The evaluation of the tabacco consumption is showed in the next tabel (Tabel no. 2).

Tabel no. 2

The evaluation of the tabacco consumption

| Tabacco consumption | Endometrial hyperplasia | | *Endometrial cancer* | |
|---|---|---|---|---|
| | No. of cases | Percentage % | No. of cases | *Percentage %* |
| Non-smokers | 325 | 52,08 % | 105 | *61,40 %* |
| In the past | 162 | 25,96 % | 48 | *28,07 %* |
| *Current* | *137* | *21,95 %* | *18* | *10,52 %* |

The smokers have a 2-fold lower risk of endometrial cancer, this effect may be linked with the fact that smokers metabolise oestrogen into less active metabolites (Popiela A et al. 2003, Pike M. C. et al. 2004).

The majority of the patients with endometrial cancer (61,40%) were non-smokers and only 10,52% were continuing smokers. The percentage of the non-smokers women was higher in comparison with patients with hyperplasia (52,08%). The percentage of the continuing somkers was higher in patient with hyperplasia (respectively 21,95%).

The results are similary with those of the two other studies. The firs one showed that 62,27% of patients with cancer were non-smokers, 30,41% of them were smokers in the past and only 9,32% of them were current smokers (Newcomer M. L. et al. 2001).

In the other study the percentage were 53,04%, 38,32% and, respectively 8,64%. (Terry P. D. et al. 2002)

The smoking has affected the trends of the cancer incidence. The endometrial cancer rates are declining from around 1925 in Denmark, France and Switzerland. In Denmark and France, trends in lung cancer mortality, a strong marker for previous tobacco consumption, have been uniformely increasing in cohorts born troughout the 20th century up to 1950. Rates are highest in Denmark among the others Member State E.U. and shifted from ranking third in 1975 to first in 1995 (Weiderpass E. & Baron J. A. 2001, Bray F. et al. 2005).

A study published by Oxford University Press in 2004 conclude that smokers have a modest reduction in endometrial cancer risk (Boyle P. et al. 2004, Viswanathan A. N. et al. 2005).

The results from the US Nurses' Healty study show a significant risk reduction in past as well as continuing smokers.

## CONCLUSIONS

The majority of the women diagnosed with endometrial cancer never used oral contraceptives and were non-smokers.

The changing prevalence of orale contraceptives among young women may influence the incidence rates of endometrial cancer in the future.

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# INFLUENCE OF TREATMENT WITH VARIOUS FUNGICIDES FROMULATIONS ON SPECIFIC PATHOGENS ATTACK TO TOMATO CROPS

Paraschivu M.[1], Partal Elena[2], Paraschivu Mirela[3], Oltenacu V. C.[4]

***Key words****:attack, pathogen, pesticide, tomato, treatment*

## ABSTRACT

*The tomato pathogens complex Phytophthora infestans, Alternaria solani and Septoria lycopersici is still causing major damage in many production areas and pesticide treatment is still one of the most effective options that can be applied especially because high levels of resistance to these pathogens in cultivated tomatoes is rare. Eight fungicide treatments applied in three different moments were evaluated for their efficacy to control Phytophthora infestans, Alternaria solani and Septoria lycopersici in Isalnia area known as an important vegetable crop pond. The regression equation indicated that for each increase of the attack degree with 1% decreases fruit yield with 264 kg/ha and for each increase of P. infestans attack on leaves with 1% increase the attack frequency on tomato fruits with 1,8%. The treatment variant V1 with all eight fungicide formulations and mixture applied at each 10 days determined the most healthy tomato plants leading to high fruit yields (85,5 t/ha).*

## INTRODUCTION

Tomato (*Solanum lycopersicum*) is an important vegetable crop worldwide. The fungal diseases such as late blight (*Phytophthora infestans*), early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*) are the most important limiting factors for tomato crop sustainability all over the world and can cause up to 100% yield losses (Nowicki et al., 2012). All these pathogens cause diseases on foliage, stems and tomato fruits, particularly when the weather is cool and rainy, with heavy dew and high humidity leading to complete defoliation of tomato plants and subsequent yield reductions (Groves and Ristaino, 2000, Foolad et al., 2008, Coolong et al., 2009). Various management practices for controlling them have been used like tolerant varieties (Chaerani and Voorrips, 2006, Foolad et al., 2008, Rodeva et al., 2009, Johnson et al., 2012), natural products and biological control (Joslin et al, 2004, Portz et al., 2009, Devi and Marimuthu, 2011, Jiang et al., 2012, Pattnaik et al., 2012), chemical control (Huggenberger et al., 2005, Shen et al., 2008, Blum et al., 2010, Patel and Chandhary, 2010) but all these management strategies could not completely and effectively control these pathogens because of their physiological differentiation and quick hereditary variation. However, effective control by planting

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resistant varieties is in many cases not possible so chemical control is still one of the most effective option. Because fungicide resistance is one of the consequences of fungicide use is necessary to avoid the situation that single fungicide is applied for a long time, preferably to use them alternately or in mixture with other not cross-resistant fungicides (Lou, 2010). Thus, the aim of the present study was to determine the efficacy of different fungicide and to exploit their synergistic interactions for controlling these tomato pathogens in Isalnita conditions.

## MATERIAL AND METHODS

During 2011 year different fungicides and mixtures have been tested for their efficiency to control tomato pathogens (*Phytophthora infestans, Alternaria solani* and *Septoria lycopersici*) using a complete split plot design with two factors and four replications. The trail factors were:

Factor A: treatment applying moment

V1- treatments every 10 days, V2 – warning treatments applied when water drops from dew or irrigation were present on plants leaves, V3- first treatment was applied to the first attack symptoms and then every 7 days, V4 – untreated control. Fungicide treatments were applied according with the following scheme (Table.1).

Table 1

The scheme of applying fungicide treatments on tomato in 2011

| Specification | Treatment and the date of | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variant | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| V1 | 15.06 | 25.06 | 12.07 | 22.07 | 2.08 | 14.08 | 24.08 | 5.09 |
| V2 | 19.06 | 2.07 | 18.07 | 7.08 | 14.08 | 26.08 | 9.09 | - |
| V3 | 8.07 | 13.08 | 21.08 | - | - | - | - | - |
| V4 | Untreated control | | | | | | | |

Factor B: fungicide treatment

Fungicide treatments were made according with the following scheme:

the 1st treatment – Folio Gold 537,5 SC (mefenoxam+clorotalonil) - 0,25%

the 2nd treatment – Drago 76 WP (cymoxanil+mancozeb) - 0,2%

the 3th treatment – Topsin 500 SC (tiofanat metil) – 0,1%

the 4th treatment – Bravo 500 SC (clorotalonil) – 0,2%

the 5th treatment – Melody Compact 49 WG (iprovaricarb+copper oxychloride) – 0,2%

the 6th treatment – Infinito 686,5 SC (fluopicolide+propamocarb chlorhidrate) – 0,14%

the 7th treatment – Ortiva (ozoxystrobin) – 0,075%

the 8th treatment – Topsin 500 SC (0,1%) + Drago 76 WP (0,2%)

Cultivated tomato variety was Buzau 4 and each plot size was 10 $m^2$. All fungicides were applied with lever operated snapback sprayers fitted with cone nozzles. Control plants were not spayed with fungicide. In all trial a rating scale of 0-11 modified after Horsfall and Barratt (1945) was used to rate individual plants for leaf spot severity.

## RESULTS AND DISCUSSION

In the climatic conditions of 2011 year the most important pathogens that have been observed in tomato in Isalnita area were *Phytophthora infestans*, *Alternaria solani*, *Septoria lycopersici.*

A large number of fungicides of different chemical classes, with different modes of action are available for the control of tomato pathogens including late blight

(*Phytophthora infestans*), early blight (*Alternaria solani*) and septoria leaf spot (*Septoria lycopersici*) (Huggenberger et al., 2005, Shen at al., 2008, Zhu et al., 2008, Blum et al., 2010, Patel and Chandhary, 2010, Godal et al., 2012, Hu et al., 2012). These fungicides differ in their activity against various developmental stages in the life cycle of tomato pathogens (Cohen and Gisi, 2007). The most effective to limiting pathogens attack was the treatment V1 when were applied eight fungicide treatments every ten days, while applying the treatments warning resulted in levels of attack degrees just below the untreated control, suggesting that preventive treatment with fungicides is more effective than treatments in warning. The highest attack degrees were recorded for untreated control as a result of *Alternaria solani* (14,56%) attack, followed by *Septoria lycopersici* (6,82%) and *Phytophthora infestans* on both fruits (5,5%) and leaves (6,67%) (Fig.1). Previous findings showed that fruit damage due to late blight (*Phytophthora infestans*) in untreated plots ranged from 1,4% to 36,8% (Walgenbach et al., 1989). Walgenbach et al. (1989) showed that late blight was easily controlled in all years with a ten day fungicide application interval. However, the treatment scheme V1 with eight fungicides applied every ten days were the most effective to *Alternaria solani* probably due to high attack degree recorded comparatively with other two pathogens.

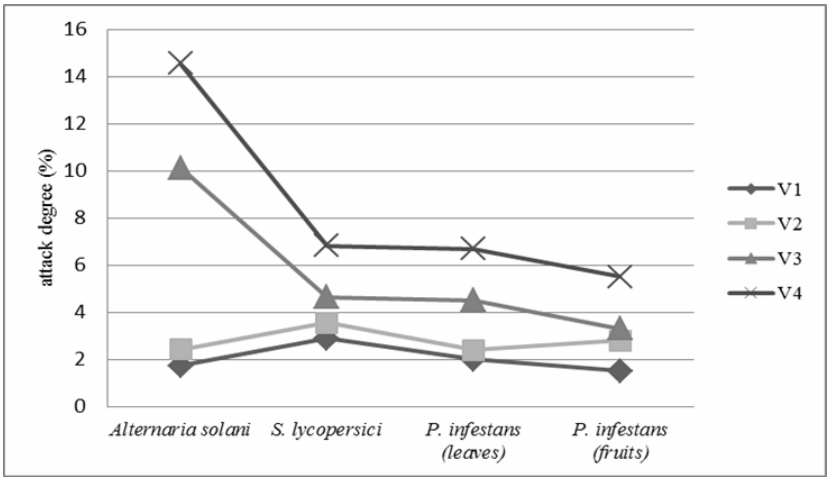


Fig. 1. The attack degrees recorded by tomato pathogens in the conditions of fungicide treatments

The efficiency of mancozeb single applied or in mixture with metalaxyl or cymoxanil to *Alternaria solani* and *Phytophthora infestance* was showed in some previous studies. Thus, Patel et al. (2010) showed that non-systemic fungicide mancozeb at 1000 ppm concentration or in mixture with metalaxyl at 1000, 1500 and 2000 ppm concentration limited *Alternaria solani* attack. Godal et al. (2012) found that mancozeb in concentration of 12 g/l water applied at an interval of 7, 14, 21 and 28 days determined the highest reduction of *Alternaria solani* attack degree. Although metalaxyl fungicide is effective, resistance to metalaxyl within populations of P. infestans has become a problem in Europe and America. The inhibitory activity of cymoxanil, mancozeb and their mixture on *Phytophthora infestans* tested at different stages in vitro showed that cymoxanil was stronger in inhibiting the mycelium growth of the pathogen than mancozeb, but it was less effective in inhibiting the germination of sporangia and zoospores. The mixture of

cymoxanil with mancozeb at a ratio of 1:7 and 1:8 were synergistic in inhibiting the germination of zoospores and mycelium growth (Wang et al., 2002). Zu et al. (2008) recommended cymoxanil and dimethomorph for controlling late blight (*Phytophthora infestans*) disease of tomato. Xiaoqiong et al. (2004) and Shen et al. (2008) showed that dimethomorph and azoxystrobin have the potential for control also late blight.

Tomato crop protection was assured especially by systemic fungicides used because they are absorbed and transported through plant tissues, making them fungitoxic, being a better alternative than contact fungicides which are easily washed by rain drops. The treatment variant V1 applied at each 10 days determined the most healthy tomato plants leading to high fruit yields (85,5 t/ha). Negative correlation between of the three pathogens attack degrees that affected tomato plants in 2011 in Isalnita was very significant (r = 0,9427***) and regression equation indicated that for each increase of the attack degree with 1% decreases fruit yield with 264 kg/ha (Fig.2).

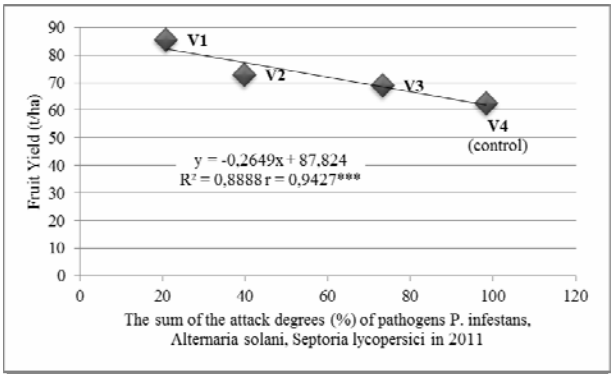


Fig. 2. Relationship between pathogens attack degrees recorded in 2011 and tomato fruit yield

Positive correlation between the attack degree of *P. infestans* on leaves and attack frequency on tomato fruits was highly significant (r = 0,9506***), suggesting that the leaf pathogen attack greatly increases the risk on pathogen attack on fruits. Thus, the regression equation indicated that for each increase of *P. infestans* attack on leaves with 1% increase the attack frequency on tomato fruits with 1,8% (Fig. 3).

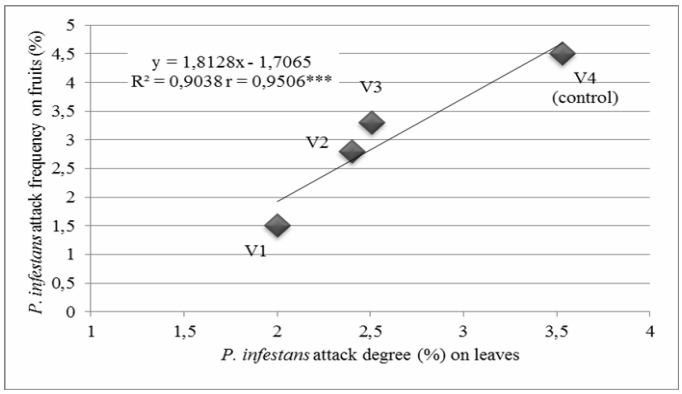


Fig.3. Relationship between the attack degree of *P. infestans* on leaves

and frequency on tomato fruits (%)

However, further investigations should be made regarding the highest fungicides efficacy and their synergistic interactions for controlling *P. infestans*, *Alternaria solani* and *Septoria lycopersici* in Isalnita area known like an important vegetable crop pond.

## CONCLUSIONS

The highest efficacy in controlling tomato pathogens complex (*Phytophthora infestans, Alternaria solani, Septoria lycopersici*) was realized when eight fungicide treatment (Folio Gold 537,5 SC (mefenoxam+clorotalonil) - 0,25%,Drago 76 WP (cymoxanil+mancozeb) - 0,2%,Topsin 500 SC (tiofanat metil) – 0,1%,Bravo 500 SC (clorotalonil) – 0,2%, Melody Compact 49 WG (iprovaricarb+copper oxychloride)-0,2%, Infinito 686,5 SC (fluopicolide+propamocarb chlorhidrate) – 0,14%, Ortiva (ozoxystrobin) – 0,075%,Topsin 500 SC (0,1%) + Drago 76 WP (0,2%) was applied at each 10 days leading to the highest fruits yield. Negative correlation between of the three pathogens attack degrees was very significant and regression equation indicated that for each increase of the attack degree with 1% decreases fruit yield with 264 kg/ha. Positive correlation between the attack degree of *P. infestans* on leaves and attack frequency on tomato fruits was highly significant and the regression equation indicated that for each increase of *P. infestans* attack on leaves with 1% increase the attack frequency on tomato fruits with 1,8%

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✓*Ingineria mediului*

**Vol. XVII ( LIII ) - 2012**

# INFLUENCE OF CULTIVAR AND PLANTING DENSITY ON THE ATTACK OF PHYTOPHTHORA INFESTANS PATHOGEN AND THE YIELD

Paraschivu Mirela[1], Paraschivu M.[2], Partal Elena[3], Oltenacu V. C.[4]

***Key words****:cultivar, Phytophthora infestans, planting density, tomato, yield*

## ABSTRACT

*Late blight (Phytophthora infestans) is one of the most devastating tomato diseases worldwide. Effective resistance is rare and fungicide application is limited in tomatoes grown in organic system. Thus, three tomato cultivars (Buzau 4, Maratonus, Siriana) grown in two different planting densities (80/30 cm and 80/40 cm) were evaluated for their response to P. infestans attack in Isalnia area known as an important vegetable crop pond. Thus, the cultivars with the lowest diseases ratings were Maratonus and Siriana, while the most affected was Buzau 4. All tomato cultivars showed the least values of disease severity for 80/40 planting density leading to the conclusion that planting density could be an effective measure to control late blight spread in tomato field. The highest yield level was recorded by the least affect cultivar suggesting that is a negatively correlation between disease severity and yield level.*

## INTRODUCTION

Tomato diseases are an ongoing problem that leads in losses of yield and quality worldwide. Practices such as the use of resistant cultivars (Johnson et al., 2012), biological products (Olanya and Larkin, 2006, Portz et al., 2009, Jiang et al., 2012), pesticide application (Blum et al., 2010) and technological measures (Tamm et al., 2010) are employed by farmers to manage and prevent economic losses caused by tomato plant diseases. The use of tolerant or resistant cultivars is a management practice recommended by both conventional and organic farmers, but high levels of resistance to pathogens in cultivated tomatoes is rare (Nowicki et al., 2012) and fungicide application can lead to pathogen resistance and environmental pollution. Thus, management of diseases in organically grown tomatoes is done by a large combination of integrated pest management practices, natural remedies and limited fungicide use (Diver et al., 1992). Late blight (*Phytophthora infestans*) is one of the most devastating tomato diseases which are difficult to control especially in organic farming. Fruit damage due to late blight (*Phytophthora infestans*) in untreated plots ranged from 1,4%-38,6% (Walgebach et al., 1989) and leaves and stems damage is much higher leading to serious losses in field (Groves and Ristaino, 2000). Furthermore, the increasing demand for organically grown products, environmental

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protection and the UE interdiction for the use of different copper-containing formulations, has precipitated an urgent need for alternative control methods. Thus, the aim of this study was to evaluate de influence of tomato cultivar and planting density to *P. infestans* severity in organic growth system.

## MATERIAL AND METHODS

Field experiment was carried out (2011) to evaluate de influence of cultivar and planting density to *Phypophthora infestans* severity using three tomato cultivars and two planting densities. The experiment was a split plot design with two factors x three replications. Each plot size was 10 $m^2$. No fungicide control was used. The trail factors were: Factor A: tomato cultivar (Buzau 4, Maratonus, Siriana) and Factor B: planting density (80/30 – 41700 pl/ha and 80/40 – 31250 pl/ha). In all trial the modified rating scale (Horsfall and Barratt, 1945) was used to rate individual plants for disease severity. The attack degree (AD %) of P. infestans was calculated for each variant according with formula AD% = (Severity% x Intensity %) /100 (Savescu et al., 1969).

## RESULTS AND DISCUSSION

Late blight (*Phypophthora infestans*) is a very difficult disease to control organically. Organic farmers should employ all integrated management strategies available (destroy all sources of inoculum, site selection, resistant cultivars, planting date, cropping system, field design, irrigation management, crop nutrition, destruction/scouting of diseased plants) to reduce late blight risk in tomato crops. However, the selection of cultivars that are adapted to local conditions or that are resistant to the pathogen is important in reducing diseases (Kokalis-Burelle, 2005). Organic growers tend to use more resistant and earlier maturing varieties than other growers. Although they may be susceptible, early varieties often escape blight epidemics (Finekh et al., 2008). Even if in 2011 year in Isalnita area the diseases pressure wasn`t so high the tomato cultivars response was different. Thus, the cultivar with the lowest diseases ratings were Maratonus and Siriana, while the most affected was Buzau 4 (Table 1). Comparatively with the control and mean value both cultivars Buzau 4 and Maratonus recorded differences statistically assured as significant.

Table 1

The influence of tomato cultivar to late blight (*P. infestans*) severity

| Cultivar | *P. infestans* AD % | Diff. control | Signif. | Diff. mean | Signif. |
|---|---|---|---|---|---|
| Siriana | 4,73 | Control | - | -0,02 | |
| Buzau 4 | 7,14 | +2,41 | * | +2,39 | * |
| Maratonus | 2,38 | -2,35 | o | -2,37 | o |
| Mean | 4,75 | | | | |
| LD 5% = 1,12, LD 1% = 2,56, LD 0,1% = 4,09 | | | | | |

These results showed that tomato susceptibility to *P. infestans* depends not only on the tomato variety, but also on the amount of pathogen inoculum available. Even cultivar choice plays a crucial role, but host resistance to late blight needs to be integrated into a complementary package of control measures. Thus, planting density influenced significantly late blight severity suggesting that little distances (80/30 cm) between tomato plants determined a higher spread of the pathogen due to closer leaves and specific microclimate that favored late blight development. The lowest diseases severity was recorded when tomato plants were planted to distance of 80/40 cm, leading to the conclusion that planting density could be an effective measure to control late blight spread in tomato field (Table 2).This aspect could be also observed for each cultivar which

recorded the lowest disease severity for planting density 80/40 cm (Table 4). Comparatively with the control (Siriana) for both planting densities the cultivars Buzau 4 and Maratonus recorded significant differences statistically assured.

Table 2

The influence of tomato planting density to late blight (*P. infestans*) severity

| Planting density | *P. infestans* AD % | Diff. control | Signif. | Diff. mean | Signif. |
|---|---|---|---|---|---|
| 80/30 cm | 5,69 | Control | - | +0,94 | |
| 40/80 cm | 3,81 | -1,88 | * | -0,94 | |
| Mean | 4,75 | | | | |
| LD 5% = 0,87, LD 1% = 1,92, LD 0,1% = 3,65 | | | | | |

Table 3

The influence of tomato cultivar x planting density to late blight (*P. infestans*) severity

| Variant | *P. infestans* AD % | Diff. control | Signif | Diff. mean | Signif |
|---|---|---|---|---|---|
| Buzau 4 (80/40) | 6,18 | +2,66 | ** | +2,37 | ** |
| Maratonus (80/40) | 1,74 | -1,78 | o | -2,07 | o |
| Siriana (80/40) | 3,52 | control | | -0,29 | |
| Mean | 3,81 | | | | |
| Buzau 4 (80/30) | 8,10 | +2,16 | * | +2,41 | ** |
| Maratonus (80/30) | 3,02 | -2,92 | oo | -2,67 | oo |
| Siriana (80/40) | 5,94 | control | | +0,25 | |
| Mean | 5,69 | | | | |
| LD 5% = 1,02, LD 1% = 2,27, LD 0,1% = 3,82 | | | | | |

The influence of late blight severity to tomato yield showed that the highest yield level was recorded by the least affect cultivar suggesting that is a negatively correlation between disease severity and yield level (Table 4). Maratonus was also the only one cultivar that proved yielding capacity up to control and mean yield.

Table 4

The influence of late blight (*P. infestans*) severity to tomato yield

| Cultivar | Total Yield (kg/m$^{)}$ | Diff. Control +Signif. | Relative yield (%) | Diff. Mean yield +Signif. | Relative yield (%) |
|---|---|---|---|---|---|
| Buzau 4 | 4,81 | -0,89 | 84,38 | -1,13$^{o}$ | 80,97 |
| Maratonus | 7,32 | +1,62$^{*}$ | 128,42 | +1,38$^{*}$ | 123,23 |
| Siriana | 5,70 | control | 100,00 | -0,24 | 95,95 |
| Mean yield | 5,94 | | | | |
| LD 5% = 1,10, LD 1% = 2,12, LD 0,1% = 3,54 | | | | | |

However, further investigations should be made regarding different technological measures that can be used for controlling *P. infestans* in tomato crops grown ecologically. Even cultivar choice and planting density have been proved usefull in decrease late blight severity these options must be integrated in a package control measures adapted to each local area conditions.

## CONCLUSIONS

Cultivar choice and planting density play an important role for controlling late blight (*Phytophthora infestans*) severity. Even if in 2011 year in Isalnita area the diseases pressure wasn`t so high, the tomato cultivars response was different, leading to the conclusion that tomato susceptibility to *P. infestans* depends not only on the tomato variety, but also on the amount of pathogen inoculum available. The cultivar with the lowest diseases ratings were Maratonus and Siriana, while the most affected was Buzau 4. The lowest diseases severity was recorded when tomato plants were planted to distance of 80/40

cm for all tested cultivars and the highest yield level was recorded by the least affect cultivar suggesting that is a negatively correlation between disease severity and yield level.

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# COLEOPTILE LENGTH AND CELLULAR EXTENSIONS VARIABILITY IN WHEAT SEEDLINGS IN WATER STRESS INDUCED CONDITIONS

Păunescu Ramona Aida[1]

***Keywords:*** *wheat, cell expansion, length coleoptile, induced water stress*

## ABSTRACT

*The experiment took place under laboratory conditions in a growth chamber that provided similar conditions as the natural environment. Water stress on the seedlings was induced using polyethylene glycol (PEG) at various concentrations.*

*The paper presents data on the coleoptile's length and the cellular extensions of the seedlings subjected to water stress. The results showed that Dropia is the only variety where induced drought hasn't changed the seedling's cellular extension level, presenting a high stability of character in the three culture environments.*

## INTRODUCTION

Water depletion is an essential issue in food security and is normally a limiting factor for plant growth at lower latitudes (Chakrabortya & Newton, 2011).

Drought is probably the most important abiotic stress limiting plant growth and crop productivity globally. The imperative to develop drought-resistant crops is intensifying due to increasingly limited water supplies for crop irrigation, in addition to stresses imposed by global climate change, such as elevated temperatures, changed precipitation patterns, and increased water deficit in arid and semiarid areas (Carolina Saint Pierre et al., 2012).

The basis to drought resistance is complex and driven by different adaptive mechanisms (Reynolds and Tuberosa, 2008), that are multigenically controlled (Blum, 2005; Pinto et al., 2010).

Morgan (1988) suggested, based on a research conducted on six F7 lines different in terms of osmotic adjustment capacity, taken from the same hybrid combination, that coleoptile length and root response to water stress can be used to identify osmoregulation differences between wheat genotypes.

Osmotic adjustment helps maintain cell turgidity by preserving water and delaying leaf wilting. This supports plant growth and productivity for resilient plants. One of the consequences in osmotic adjustments, with respect to the whole plant, is further root growth and water uptake from the soil. (Blum, http://www.plantstress.com)

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## MATERIALS AND METHODS

The chosen material includes 50 wheat genotypes from Romania and other countries, selected to represent several different wheat ecotypes. This collection consists of 23 Romanian varieties with different drought performance and other foreign varieties from Austria, France, Germany, Bulgaria, Israel, Hungary, Turkey, Serbia and Russia.

The analysis is divided in two experiments, as follows:

**Experiment 1** - bifactorial expertise studying coleoptile length, where:

**Factor A –** variety with 50 graduations ( tabel 1);

**Factor B –** the culture medium with 2 graduation: $H_2O$ (witness) and 25% PEG 10000 (plantlets subjected to induced water stress)

**Experiment 2 -** bifactorial expertise studying cellular extension of plantlets, where:

**Factor A –** variety with 50 graduations (tabel 1);

**Factor B –** the culture medium with 3 graduation: $H_2O$ (witness), 25% PEG 10000 and 40% PEG10000 (plantlets subjected to induced water stress).

Research method: 50 seeds of each genotype were germinated in Petri dishes in two culture media: water and 25% PEG 10000. After 10 days the coleoptile length was measured at 6 seedlings in each culture medium. 18 seedlings from each wheat genotype used as control (water) were transferred in rolls of 6 seedlings each and placed in the growth chamber. Each genotype received three culture media: $H_2O$, 25% PEG 10000 and 40% PEG 4000. After 30 days, each plantlet was measured to determine the cellular extension of the genotypes in the three cell culture media.

PEG was used to induce water stress on the seedlings. Blum (2005) showed that PEG can be used to modify the osmotic potential of a culture in nutrient solution by inducing a fluid stress to plants in a controlled manner, similar to natural conditions.

## RESULTS AND DISCUSSIONS

The variability coefficient of the coleoptile length ranged from 4.49% to 25.19% for the Şimnic 30 variety in the water medium. When the plant was grown in PEG 10000, the variability coefficient reached up to 76.44% for Romansa variety.

Dor ,Glosa and Mv. Palma are the only varieties that slightly modified their coleoptile length depending on the substrate, therefore showing a high stability to drought during sunrise.

Delabrad , Eliana, Oratorio, Nathan and Shoham varieties didn't germinate when subjected to induced drought, suggesting that they are sensitive to drought occurring during the germination period (table 1).

Aztec, Carolina, Crina, Gabriela, GK Gobe, GK David, Miranda, GK Hatyyu, Lovrin 34, Orqual were varieties that showed very high stability of the studied character, when the culture medium was water.

In regards to the seedling length, Dropia is the only variety where induced drought doesn't alter the seedlings degree of cellular extension, this character presenting a high stability in all three culture media.

Depending on the results, there are several types of varieties, as follows:

- the varieties that change in a very small degree in cell extension in the three culture media are: Alex, Lilies, Dariel, Miranda, Moldau and Oratorio.
- the varieties that show no change in seedlings length at moderate induced water stress are: Aztec, Boema, Miss, Dropia, Gabriela, GK Hattyu, Izvor and Nikifor.

-the varieties that show no change in seedlings length at high intensity induced water stress are: Agron, Bitop, Capo, Carolina, Dropia, Faur, Flamura and Nathan.
-varieties that changed in a very small degree in the seedling's cell extension at high intensity induced water stress are: Alex, Aztec, Boema, Crina, Dariel, Delabrad, Dor, Dunai, Eliana, Enesco, Esquisse, Exotic, Gabriela, GK Hatty Miranda, Izvor, Moldau, Mv Palma, Nikifor, Oratrio, Romulus and Shoham (table 1).

Demetra variety, though having a high variability, changed its extension degree independently to the cell culture media.

Esquisit , Julius and Simnic 30 varieties did not last till the end of the experiment.

Table 1

Variability coefficient– coleoptile and plantlet length

| No. | Variety | Condition | Variability coefficient- length coleoptile | | Variability coefficient– plantlet length | |
|---|---|---|---|---|---|---|
| | | | $x \square \pm s_x$ | S% | $x \square \pm s_x$ | S% |
| 1. | AGRON | $H_2O$ | 24,98±2,5 | 10,02 | 7,30±0,92 | 12,61 |
| | | 25% PEG 10000 | 21,54±2,43 | 11,28 | 1,97±0,95 | 48,10 |
| | | 40% PEG 4000 | 24,08±1,49 | 6,17 | | |
| 2. | ALEX | $H_2O$ | 16,88±2,33 | 13,83 | 8,25±1,33 | 16,09 |
| | | 25% PEG 10000 | 20,43±3,15 | 15,40 | 0,57±0,20 | 34,71 |
| | | 40% PEG 4000 | 18,88±3,43 | 18,14 | | |
| 3. | AZTEC | $H_2O$ | 20,57±3,82 | 18,57 | 7,48±0,71 | 9,50 |
| | | 25% PEG 10000 | 20,78±1,24 | 5,97 | 0,15±0,05 | 36,51 |
| | | 40% PEG 4000 | 16,10±1,93 | 11,97 | | |
| 4. | BEZOSTAIA | $H_2O$ | 21,26±2,41 | 11,32 | 10,33±1,25 | 12,07 |
| | | 25% PEG 10000 | 19,95±3,23 | 16,21 | 0,48±0,23 | 47,94 |
| | | 40% PEG 4000 | 18,80±4,78 | 25,43 | | |
| 5. | BITOP | $H_2O$ | 20,38±1,82 | 8,95 | 8,05±1,20 | 14,96 |
| | | 25% PEG 10000 | 20,38±5,11 | 25,06 | 0,38±0,12 | 30,51 |
| | | 40% PEG 4000 | 23,03±1,73 | 7,52 | | |
| 6. | BOEMA | $H_2O$ | 20,38±1,82 | 8,95 | 8,11±1,77 | 21,78 |
| | | 25% PEG 10000 | 19,37±1,53 | 7,88 | 1,02±0,43 | 41,93 |
| | | 40% PEG 4000 | 15,93±3,03 | 19,06 | | |
| 7. | ŞIMNIC 50 | $H_2O$ | 21,00±1,49 | 7,09 | 7,65±0,88 | 11,54 |
| | | 25% PEG 10000 | 18,60±5,07 | 27,24 | 2,58±0,76 | 29,32 |
| | | 40% PEG 4000 | 19,37±4,04 | 20,85 | | |
| 8. | CAPO | $H_2O$ | 19,48±3,16 | 16,22 | 8,47±1,28 | 15,12 |
| | | 25% PEG 10000 | 19,42±3,93 | 20,22 | 3,12±0,85 | 27,26 |
| | | 40% PEG 4000 | 15,10±1,25 | 8,27 | | |
| 9. | CAROLINA | $H_2O$ | 19,62±3,64 | 18,56 | 8,33±0,75 | 8,97 |
| | | 25% PEG 10000 | 19,06±2,19 | 11,47 | 0,12±0,04 | 35,13 |
| | | 40% PEG 4000 | 17,72±1,55 | 8,76 | | |
| 10. | CRINA | $H_2O$ | 18,73±3,61 | 19,28 | 6,85±0,57 | 8,30 |
| | | 25% PEG 10000 | 19,00±2,68 | 14,10 | 0,28±0,13 | 46,93 |
| | | 40% PEG 4000 | 16,77±2,98 | 17,76 | | |
| 11. | CUBUS | $H_2O$ | 17,98±4,43 | 24,67 | 8,08±0,05 | 13,58 |
| | | 25% PEG 10000 | 17,30±3,20 | 18,52 | 0,15±0,05 | 36,51 |
| | | 40% PEG 4000 | 15,37±4,38 | 28,49 | | |
| 12. | DARIEL | $H_2O$ | 15,20±2,69 | 17,72 | 7,48±1,12 | 14,99 |
| | | 25% PEG 10000 | 9,92±1,81 | 18,29 | 0,15±0,05 | 36,51 |
| | | 40% PEG 4000 | 15,98±2,84 | 17,76 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 13. | DELABRAD | $H_2O$ | 22,20±2,16 | 9,72 | 8,02±1,19 | 14,83 |
| | | 25% PEG 10000 | 23,26±2,84 | 12,19 | 0,2±0 | 0 |
| | | 40% PEG 4000 | 22,47±3,82 | 17,01 | | |
| 14. | DEMETRA | $H_2O$ | 21,20±4,78 | 22,54 | 11,75±1,62 | 13,83 |
| | | 25% PEG 10000 | 20,85±6,50 | 31,15 | 0,15±0,05 | 36,51 |
| | | 40% PEG 4000 | 17,28±3,64 | 21,05 | | |
| 15. | DOR | $H_2O$ | 18,10±4,15 | 22,94 | 8,45±0,98 | 11,61 |
| | | 25% PEG 10000 | 23,42±2,27 | 9,69 | 2,63±0,34 | 12,86 |
| | | 40% PEG 4000 | 20,78±2,47 | 11,89 | | |
| 16. | DROPIA | $H_2O$ | 21,13±1,04 | 4,94 | 7,82±1,09 | 13,98 |
| | | 25% PEG 10000 | 21,48±1,92 | 8,95 | 0,22±0,08 | 34,78 |
| | | 40% PEG 4000 | 17,44±1,43 | 8,20 | | |
| 17. | DUNAI | $H_2O$ | 19,17±5,09 | 26,53 | 9,93±1,24 | 12,46 |
| | | 25% PEG 10000 | 17,92±3,07 | 17,15 | 0,25±0,05 | 21,91 |
| | | 40% PEG 4000 | 16,92±2,47 | 14,62 | | |
| 18. | ELIANA | $H_2O$ | 19,35±5,05 | 26,09 | 7,05±1,09 | 15,53 |
| | | 25% PEG 10000 | 18,20±4,19 | 23,03 | 0,1±0 | 0 |
| | | 40% PEG 4000 | 19,20±3,53 | 18,41 | | |
| 19. | ENESCO | $H_2O$ | 18,40±3,69 | 20,06 | 6,32±0,79 | 12,52 |
| | | 25% PEG 10000 | 20,93±3,96 | 18,89 | 0,37±0,15 | 41,07 |
| | | 40% PEG 4000 | 15,80±2,52 | 15,97 | | |
| 20. | ESQUISIT | $H_2O$ | 20,30±1,98 | 9,75 | 6,5±0,90 | 13,83 |
| | | 25% PEG 10000 | - | - | 0,42±0,15 | 35,34 |
| | | 40% PEG 4000 | 19,03±3,00 | 15,76 | | |
| 21. | EXOTIC | $H_2O$ | 18,35±3,96 | 21,61 | 6,22±0,65 | 10,45 |
| | | 25% PEG 10000 | 17,48±4,77 | 27,26 | 0,15±0,05 | 36,51 |
| | | 40% PEG 4000 | 12,37±1,80 | 14,54 | | |
| 22. | FAUR | $H_2O$ | 23,48±3,66 | 15,61 | 6,28±1,06 | 16,86 |
| | | 25% PEG 10000 | 22,20±2,90 | 13,04 | 0,12±0,04 | 35,13 |
| | | 40% PEG 4000 | 20,10±1,60 | 7,94 | | |
| 23. | FLAMURA | $H_2O$ | 17,68±4,29 | 24,29 | 9,22±0,99 | 10,73 |
| | | 25% PEG 10000 | 19,13±3,74 | 19,57 | 2,17±0,47 | 21,58 |
| | | 40% PEG 4000 | 17,97±1,31 | 7,26 | | |
| 24. | GABRIELA | $H_2O$ | 21,22±0,88 | 4,12 | 7,05±0,61 | 8,60 |
| | | 25% PEG 10000 | 19,60±1,91 | 9,73 | 0,15±0,05 | 36,51 |
| | | 40% PEG 4000 | 19,42±3,43 | 17,67 | | |
| 25. | GIAVA | $H_2O$ | 21,92±3,24 | 14,79 | 7,48±0,88 | 11,72 |
| | | 25% PEG 10000 | 16,34±3,11 | 19,03 | 0,15±0,08 | 55,78 |
| | | 40% PEG 4000 | 16,04±5,33 | 33,20 | | |
| 26. | GK DAVID | $H_2O$ | 18,43±2,36 | 12,83 | 7,52±0,34 | 4,49 |
| | | 25% PEG 10000 | 17,64±4,36 | 24,71 | 0,13±0,05 | 38,83 |
| | | 40% PEG 4000 | 13,52±3,32 | 24,53 | | |
| 27. | GK ELET | $H_2O$ | 21,55±2,07 | 9,59 | 9,50±1,19 | 12,53 |
| | | 25% PEG 10000 | 17,56±3,39 | 19,28 | 2,43±0,58 | 23,63 |
| | | 40% PEG 4000 | 14,84±4,99 | 33,63 | | |
| 28. | GLOSA | $H_2O$ | 21,68±2,67 | 12,32 | 8,30±1,53 | 18,38 |
| | | 25% PEG 10000 | 18,43±3,26 | 17,67 | 3,47±0,40 | 11,49 |
| | | 40% PEG 4000 | 17,22±3,75 | 21,76 | | |
| 29. | GK GOBE | $H_2O$ | 19,75±2,62 | 13,25 | 9,35±0,84 | 9,02 |
| | | 25% PEG 10000 | 24,76±2,65 | 10,72 | 2,00±0,89 | 44,61 |
| | | 40% PEG 4000 | 15,63±6,75 | 43,20 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 30. | GRUIA | $H_2O$ | 19,94±4,57 | 22,90 | 8,38±1,24 | 14,79 |
| | | 25% PEG 10000 | 18,68±2,49 | 13,34 | 1,45±0,89 | 61,34 |
| | | 40% PEG 4000 | 16,52±3,90 | 23,64 | | |
| 31. | GK HATTYU | $H_2O$ | 18,72±4,05 | 21,66 | 7,93±0,53 | 6,70 |
| | | 25% PEG 10000 | 16,50±1,55 | 9,40 | 1,45±0,89 | 61,34 |
| | | 40% PEG 4000 | 15,07±1,71 | 11,37 | | |
| 32. | MIRANDA | $H_2O$ | 21,20±4,13 | 19,50 | 8,02±0,80 | 9,93 |
| | | 25% PEG 10000 | 18,22±3,50 | 19,24 | 0,12±0,04 | 35,13 |
| | | 40% PEG 4000 | 18,77±2,24 | 11,91 | | |
| 33. | IZVOR | $H_2O$ | 26,37±1,05 | 3,98 | 7,42±1,00 | 13,47 |
| | | 25% PEG 10000 | 21,60±2,08 | 9,62 | 0,42±0,23 | 55,61 |
| | | 40% PEG 4000 | 21,22±2,66 | 12,54 | | |
| 34. | KARLYGASA | $H_2O$ | 23,50±3,81 | 16,20 | 8,22±1,28 | 15,54 |
| | | 25% PEG 10000 | 17,80±2,48 | 13,96 | 2,27±1,33 | 58,84 |
| | | 40% PEG 4000 | 15,22±5,16 | 33,88 | | |
| 35. | KRISTINA | $H_2O$ | 20,82±3,79 | 18,19 | 5,62±0,94 | 16,68 |
| | | 25% PEG 10000 | 14,20±2,85 | 20,10 | 2,9±0,65 | 22,35 |
| | | 40% PEG 4000 | 14,64±3,41 | 23,29 | | |
| 36. | LADA | $H_2O$ | 21,23±5,01 | 23,60 | 7,68±1,26 | 16,45 |
| | | 25% PEG 10000 | 17,42±2,42 | 13,90 | 0,50±0,23 | 45,61 |
| | | 40% PEG 4000 | 18,63±5,45 | 29,25 | | |
| 37. | LITERA | $H_2O$ | 29,25±1,91 | 6,53 | 6,97±1,17 | 16,78 |
| | | 25% PEG 10000 | 21,47±4,32 | 20,12 | 0,13±0,05 | 38,83 |
| | | 40% PEG 4000 | 14,63±6,50 | 44,45 | | |
| 38. | LOVRIN 34 | $H_2O$ | 25,70±1,17 | 4,54 | 6,82±0,53 | 7,84 |
| | | 25% PEG 10000 | 18,32±3,02 | 16,51 | 1,27±0,58 | 45,67 |
| | | 40% PEG 4000 | 16,13±4,74 | 29,37 | | |
| 39. | MOLDAU | $H_2O$ | 22,08±2,40 | 10,88 | 9,55±1,35 | 14,15 |
| | | 25% PEG 10000 | 12,68±2,20 | 17,36 | 0,13±0,05 | 38,83 |
| | | 40% PEG 4000 | 14,37±2,28 | 15,88 | | |
| 40. | MV PALMA | $H_2O$ | 23,32±2,21 | 9,52 | 7,5±0,86 | 11,53 |
| | | 25% PEG 10000 | 18,63±5,90 | 31,68 | 3,98±0,31 | 7,85 |
| | | 40% PEG 4000 | 19,55±2,86 | 14,63 | | |
| 41. | NIKIFOR | $H_2O$ | 21,87±3,16 | 14,47 | 8,5±1,60 | 18,84 |
| | | 25% PEG 10000 | 25,43±2,34 | 9,21 | 0,22±0,12 | 53,98 |
| | | 40% PEG 4000 | 18,90±3,78 | 20,01 | | |
| 42. | ORATORIO | $H_2O$ | 21,17±3,62 | 17,12 | 8,47±1,04 | 12,24 |
| | | 25% PEG 10000 | 17,08±1,86 | 10,86 | 0,1±0 | 0 |
| | | 40% PEG 4000 | 15,90±2,12 | 13,33 | | |
| 43. | ORQUAL | $H_2O$ | 23,47±2,61 | 11,14 | 8,17±0,69 | 8,44 |
| | | 25% PEG 10000 | 20,65±2,14 | 10,34 | 0,28±0,15 | 51,97 |
| | | 40% PEG 4000 | 14,42±4,29 | 29,79 | | |
| 44. | JULIUS | $H_2O$ | 22,56±1,87 | 8,27 | 7,27±0,64 | 8,86 |
| | | 25% PEG 10000 | 17,35±4,32 | 24,87 | 0,88±0,62 | 70,67 |
| | | 40% PEG 4000 | - | - | | |
| 45. | NATHAN | $H_2O$ | 19,13±1,94 | 10,14 | 8,82±0,60 | 6,82 |
| | | 25% PEG 10000 | 15,88±1,96 | 12,32 | 0,1±0 | 0 |
| | | 40% PEG 4000 | 13,00±1,15 | 8,88 | | |
| 46. | PKB ROMANSA | $H_2O$ | 20,43±3,04 | 14,90 | 8,25±0,92 | 11,16 |
| | | 25% PEG 10000 | 19,87±4,05 | 20,36 | 1,33±1,02 | 76,44 |
| | | 40% PEG 4000 | 18,07±5,02 | 27,79 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 47. | ROMULUS | $H_2O$ | 25,40±7,35 | 28,95 | 7,85±1,06 | 13,50 |
| | | 25% PEG 10000 | 19,78±7,17 | 36,26 | 2,13±0,77 | 35,90 |
| | | 40% PEG 4000 | 15,54±2,67 | 17,18 | | |
| 48. | SHOHAM | $H_2O$ | 16,24±3,57 | 22,00 | 7,95±1,60 | 20,15 |
| | | 25% PEG 10000 | 18,55±4,29 | 23,13 | 0,1±0 | 0 |
| | | 40% PEG 4000 | 12,75±2,53 | 19,87 | | |
| 49. | SIMNIC 30 | $H_2O$ | 21,77±4,50 | 20,67 | 9,45±2,38 | 25,19 |
| | | 25% PEG 10000 | 17,33±5,01 | 28,89 | 0,22±0,04 | 18,92 |
| | | 40% PEG 4000 | - | - | | |
| 50. | TRIVALE | $H_2O$ | 22,95±3,81 | 16,58 | 9,03±1,10 | 12,14 |
| | | 25% PEG 10000 | 17,27±3,49 | 20,20 | 2,48±1,36 | 54,83 |
| | | 40% PEG 4000 | 17,43±4,07 | 23,37 | | |

## CONCLUSION

Dor, Glosa and Mv. Palma varieties are the only ones that slightly modified their coleoptile length depending on the substrate, therefore showing a high stability to drought during sunrise.

During the induction of water stress, using PEG 10,000, the variability coefficient can change the coleoptile's length up to 76.44%.

From the results of the two experiments, we can observe that Oratorio variety is very sensitive to drought, when it occurs during germination period; however, when it occurs during the vegetation period, it has a high stability to drought.

Dropia is the only variety where induced drought doesn't alter the degree of the seedling's cellular extension, presenting a high stability of the character in all three culture media.

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# CHARACTERIZATION OF WHEAT VARIETIES REGARDING FOLIAGE AND WATER LOSS VELOCITY OF THE FLAG LEAF, IN THE SIMNIC'S LUVOSOILS

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***Key words*:** *wheat, foliage surface, water loss capacity, drought resistance*

## ABSTRACT

*Wheat production under drought conditions depends on many specific physiological traits that directly or indirectly determine the plants response to hydric deficit. The plant's survival capacity, in rough drought conditions, depends on its capacity to diminish water loss trough leaves, after the stomata reach a minimum opening. The plant's water use efficiency is usually equivalent to its drought resistance. In this way, the foliage surface and capacity for water loss are important in characterizing wheat genotypes regarding their capacity to withstand drought conditions. The foliage surface limits for the Romanian varieties ranged between 1226 and 2558 mm$^2$ and for the foreign varieties between 1194 and 2785 mm$^2$. Varieties that lost the most water in the first 4 hours after the leaves were excised lost less water in the the next 20 hours of withering.*

## INTRODUCTION

Water stress is one of the most limiting environmental factors to plant productivity worldwide, and can be caused by both soil and atmospheric water deficits. (Oula Ghannoum, 2009)

Varieties performance, under drought conditions, depends on several characters but none of them can explain by themselves the observed differences of genotype behavior. (Monica David, 2012).

Many physiological and morphological strategies have been identified in response to water deficit ranging from dehydration avoidance to dehydration tolerance. The plant 'avoids' the stress by different strategies that include deep rooting (Lopes & Reynolds, 2010), reduced leaf area, reduced growth duration (early flowering), and mechanisms related to increased water use efficiency (WUE) (Araus et al., 2002).

WUE has been defined as the ratio of total biomass or above-ground biomass or grain yield and water used during crop growth (Angus & van Herwarden, 2001). Under drought, grain yield can be expressed in relation to water use (WU), WUE, and harvest index (HI) (grain yield¼WU3WUE3HI; Salekdeh et al., 2009).

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Clarke, in 1992, proposed that the low rate of water loss (RWL) from excised leaves was a screening method of wheat cultivars (*Triticum* sp.) with better resistance to drought.

Petcu et al., in 2007, had shown that in drought conditions, the foliage surface are reduced simultaneously time with the leaves number; therefore the plant limits its water consumption.

## MATERIALS AND METHODS

The experiment consisted in cropping 50 wheat genotypes from Romania and other countries, particularly selected to represent different ecotypes. The experimental field was located in the Plant Amelioration sector of A.D.R.S. Simnic. The analysis was made in 50 versions with three repetitions and it was set up as a triple balanced triangle repeating the main base.

The foliage surface and water loss velocity, of the excised leaves, was determined in the laboratory by using the Area Meter 300 device.

For determining the foliage surface, in the experimental field, two flag leaves from each genotype of each repetition were excised and introduced in labeled cellophane wraps that were transported shortly after into the laboratory and stored in the refrigerator for further analysis with the Area Meter 300.

Water loss velocity measurements of the flag leaves consist of 6 leaves from each variety beings excised from each plant variety in the experiment; the leaves were transported in less than 30 minutes to the laboratory and weighted for determining the initial water content (IWC). Then, the leaves were dried for 4 hours in laboratory conditions (20ºC, in the dark) and then weighted for obtaining the $W_{h4.}$ The water lost by drying, after 4 hours was calculated using the formula:

$WL_{4h}=(IWC-W_{4h})/DW$

Then the leaves were dryed for another 20 hours at 20ºC and re-weighted for obtaining the $W_{24h.}$ Finally, the leaves were dried in the sterilizing room at 70ºC in order to obtain the dry weight. The water loss between 4 and 24 hours was estimated using the formula:

$WL4\text{-}24h=(W_{4h}-W_{24h})/DW$

Where: DW= dry weight

IWC= initial water content

$WL_{24}$ and $WL_4$= water loss after four and 24 hours.

Foto 1: Aspects during the analysis

## RESULTS AND DISCUSSIONS

The lowest value of the foliage surface was registered for the Oratorio variety and the highest value was found for the Hattyu variety.

The limits of foliage surface for Romanian varieties ranged between 1226 and 2558 $mm^2$, and for the foreign varieties the values were between 1194 and 2785 $mm^2$. The varieties presented statistically interpretable surface foliage values after we reference it to Dropia and also when we referenced it to the average value. When Dropia witness was taken as reference, the following varieties showed statistically proven decreases: Agron, Boema, Carolina, Cubus, Dunai, Giava, GK Gobe, Miranda, Izvor, Litera, Lv 34, Moldau, Nikifor, Oratorio, Julius, Nathan, Romulus şi Shoham.

Moldau, Oratorio and Romulus presented significant reductions of the foliage area compared to the Dropia variety.

Gk Hattyu is the only variety that presented a significat increase in foliage area after comparing it with the Dropia witness.

With respect to the average, there are several variety categories with registered surface foliage value, as follows:

- Varieties that registered significant decreases: Izvor, Moldau, Nikifor, Orarorio, Julius, Nathan, Romulus, Shoham;
- Varieties that registered significant increases: Dariel, Delabrad, Flamura, GK Elet and Gruia
- Varieties that registered very significant increases: Gk Hattyu and Kristina

Table 1

Data regarding the foliage area of the flag leaf

| No. | Genotype | Foliar surface ($mm^2$) | Dropia witness | | Average | |
|---|---|---|---|---|---|---|
| | | | Dif. | Signif. | Dif. | Signif. |
| 1. | AGRON | 1660 | -554 | o | -221 | |
| 2. | ALEX | 1982 | -232 | | 101 | |
| 3. | AZTEC | 1937 | -277 | | 56 | |
| 4. | BEZOSTAIA | 2340 | 126 | | 459 | |
| 5. | BITOP | 1861 | -353 | | -20 | |
| 6. | BOEMA | 1619 | -595 | o | -262 | |
| 7. | BRIANA | 2107 | -107 | | 226 | |
| 8. | CAPO | 1810 | -404 | | -71 | |
| 9. | CAROLINA | 1587 | -627 | o | -294 | |
| 10. | CRINA | 1992 | -222 | | 111 | |
| 11. | CUBUS | 1585 | -629 | o | -296 | |
| 12. | DARIEL | 2525 | 311 | | 644 | * |
| 13. | DELABRAD | 2442 | 228 | | 561 | * |
| 14. | DEMETRA | 1834 | -380 | | -47 | |
| 15. | DOR | 1992 | -222 | | 111 | |
| 16. | DROPIA | 2214 | 0 | | 333 | |
| 17. | DUNAI | 1485 | -729 | oo | -396 | |
| 18. | ELIANA | 1931 | -283 | | 50 | |
| 19. | ENESCO | 1994 | -220 | | 113 | |
| 20. | ESQUISIT | 1766 | -448 | | -115 | |
| 21. | EXOTIC | 2061 | -153 | | 180 | |
| 22. | FAUR | 1943 | -271 | | 62 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 23. | FLAMURA | 2558 | 344 | | 677 | * |
| 24. | GABRIELA | 2019 | -195 | | 138 | |
| 25. | GIAVA | 1694 | -520 | o | -187 | |
| 26. | GK DAVID | 2008 | -206 | | 127 | |
| 27. | GK ELET | 2462 | 248 | | 581 | * |
| 28. | GLOSA | 2162 | -52 | | 281 | |
| 29. | GK GOBE | 1684 | -530 | o | -197 | |
| 30. | GRUIA | 2472 | 258 | | 591 | * |
| 31. | GK HATTYU | 2785 | 571 | * | 904 | ** |
| 32. | MIRANDA | 1693 | -521 | o | -188 | |
| 33. | IZVOR | 1360 | -854 | oo | -521 | o |
| 34. | KARLYGASA | 2296 | 82 | | 415 | |
| 35. | KRISTINA | 2696 | 482 | | 815 | ** |
| 36. | LADA | 2123 | -91 | | 242 | |
| 37. | LITERA | 1402 | -812 | oo | -479 | |
| 38. | LOVRIN 34 | 1546 | -668 | o | -335 | |
| 39. | MOLDAU | 1204 | -1010 | ooo | -677 | o |
| 40. | MV PALMA | 1908 | -306 | | 27 | |
| 41. | NIKIFOR | 1349 | -865 | oo | -532 | o |
| 42. | ORATORIO | 1194 | -1020 | ooo | -687 | o |
| 43. | ORQUAL | 2030 | -184 | | 149 | |
| 44. | JULIUS | 1349 | -865 | oo | -532 | o |
| 45. | NATHAN | 1273 | -941 | oo | -608 | o |
| 46. | PKB ROMANSA | 1995 | -219 | | 114 | |
| 47. | ROMULUS | 1226 | -988 | ooo | -655 | o |
| 48. | SHOHAM | 1362 | -852 | oo | -519 | o |
| 49. | SIMNIC 30 | 1784 | -430 | | -97 | |
| 50. | TRIVALE | 1735 | -479 | | -146 | |
| | DL 5% | | 520 | | | |
| | DL 1% | | 705 | | | |
| | DL 0,1% | | 944 | | | |

For both determinations, after 4 and 24 h, when we compared them to the average, the varieties showed statistically proven differences regarding the water loss velocity.

Dariel and Dropia varieties registered very significant reductions of the water loss velocity after a 4 hour of whithering. The Nathan variety registered a significant reductions of the water loss velocity after a 4 hour whithering, but after another 20h it registered a significant increase. The varieties that registered a significant increase in water loss velocity after four hours were: Glosa and GK Gobe.

Capo variety registered a highly significant increase in the water loss velocity after the first 4 hours. Gruia variety registered a highly significant increase in water loss velocity, but after 20 hours of withering, it registered a significant decrease.

PKB Romansa variety had a highly significant decrease in water loss velocity after the first 4 hours and in the next 20 hours had a significant decrease. According to the obtained results, there are more variety categories regarding water loss velocity between four and 24 h of withering, as follows:

- varieties that registered significant decreases: Bezostaia, Delabrad and PKB Romansa;
- varieties that registered highly significant decreases: Gruia and Simnic 30;
- varieties that registered significant increases: Boema, Cubic and Nathan.

Table 2

Data regarding water loss velocity from the flag leaf

| No. | Genotype | WL4h=(IWC-W4h)/DW With respect to the Average | | | WL4-24h=(W4h-W24h)/DW With respect to the Average | | |
|---|---|---|---|---|---|---|---|
| | | Value | Dif. | Signif. | Value | Dif. | Signif. |
| 1. | AGRON | 0,765 | 0,102 | | 0,554 | 0,077 | |
| 2. | ALEX | 0,636 | -0,028 | | 0,577 | 0,100 | |
| 3. | AZTEC | 0,804 | 0,141 | | 0,497 | 0,020 | |
| 4. | BEZOSTAIA | 0,777 | 0,114 | | 0,324 | -0,154 | o |
| 5. | BITOP | 0,610 | -0,053 | | 0,510 | 0,033 | |
| 6. | BOEMA | 0,711 | 0,048 | | 0,683 | 0,206 | * |
| 7. | BRIANA | 0,639 | -0,024 | | 0,449 | -0,028 | |
| 8. | CAPO | 0,966 | 0,303 | ** | 0,391 | -0,086 | |
| 9. | CAROLINA | 0,571 | -0,093 | | 0,529 | 0,052 | |
| 10. | CRINA | 0,659 | -0,005 | | 0,576 | 0,099 | |
| 11. | CUBUS | 0,600 | -0,063 | | 0,688 | 0,211 | * |
| 12. | DARIEL | 0,332 | -0,331 | oo | 0,431 | -0,047 | |
| 13. | DELABRAD | 0,798 | 0,135 | | 0,319 | -0,158 | o |
| 14. | DEMETRA | 0,736 | 0,073 | | 0,589 | 0,112 | |
| 15. | DOR | 0,666 | 0,002 | | 0,608 | 0,131 | |
| 16. | DROPIA | 0,394 | -0,270 | oo | 0,379 | -0,098 | |
| 17. | DUNAI | 0,837 | 0,174 | | 0,350 | -0,128 | |
| 18. | ELIANA | 0,778 | 0,115 | | 0,525 | 0,048 | |
| 19. | ENESCO | 0,732 | 0,069 | | 0,543 | 0,066 | |
| 20. | ESQUISIT | 0,641 | -0,023 | | 0,534 | 0,057 | |
| 21. | EXOTIC | 0,691 | 0,028 | | 0,484 | 0,007 | |
| 22. | FAUR | 0,685 | 0,022 | | 0,526 | 0,049 | |
| 23. | FLAMURA | 0,820 | 0,157 | | 0,509 | 0,032 | |
| 24. | GABRIELA | 0,816 | 0,153 | | 0,408 | -0,069 | |
| 25. | GIAVA | 0,545 | -0,119 | | 0,534 | 0,057 | |
| 26. | GK DAVID | 0,545 | -0,119 | | 0,402 | -0,076 | |
| 27. | GK ELET | 0,695 | 0,032 | | 0,488 | 0,011 | |
| 28. | GLOSA | 0,867 | 0,204 | * | 0,452 | -0,026 | |
| 29. | GK GOBE | 0,862 | 0,199 | * | 0,512 | 0,035 | |
| 30. | GRUIA | 1,180 | 0,517 | *** | 0,219 | -0,258 | oo |
| 31. | GK HATTYU | 0,532 | -0,132 | | 0,558 | 0,081 | |
| 32. | MIRANDA | 0,688 | 0,025 | | 0,558 | 0,081 | |
| 33. | IZVOR | 0,633 | -0,030 | | 0,559 | 0,082 | |
| 34. | KARLYGASA | 0,736 | 0,073 | | 0,531 | 0,054 | |
| 35. | KRISTINA | 0,555 | -0,109 | | 0,562 | 0,085 | |
| 36. | LADA | 0,633 | -0,030 | | 0,543 | 0,066 | |
| 37. | LITERA | 0,504 | -0,159 | | 0,436 | -0,042 | |
| 38. | LOVRIN 34 | 0,661 | -0,002 | | 0,457 | -0,021 | |
| 39. | MOLDAU | 0,578 | -0,085 | | 0,471 | -0,006 | |
| 40. | MV PALMA | 0,711 | 0,048 | | 0,348 | -0,130 | |
| 41. | NIKIFOR | 0,662 | -0,002 | | 0,356 | -0,121 | |
| 42. | ORATORIO | 0,526 | -0,137 | | 0,512 | 0,035 | |
| 43. | ORQUAL | 0,549 | -0,114 | | 0,414 | -0,063 | |
| 44. | JULIUS | 0,467 | -0,197 | | 0,474 | -0,003 | |
| 45. | NATHAN | 0,474 | -0,189 | o | 0,633 | 0,156 | * |
| 46. | PKB ROMANSA | 0,303 | -0,360 | ooo | 0,303 | -0,174 | o |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 47. | ROMULUS | 0,716 | 0,053 | | 0,394 | -0,083 | |
| 48. | SHOHAM | 0,662 | -0,002 | | 0,463 | -0,014 | |
| 49. | SIMNIC 30 | 0,598 | -0,066 | | 0,248 | -0,229 | oo |
| 50. | TRIVALE | 0,628 | -0,035 | | 0,435 | -0,043 | |
| | DL 5% | 0,186 | | | 0,153 | | |
| | DL 1% | 0,252 | | | 0,207 | | |
| | DL 0,1% | 0,338 | | | 0,278 | | |

## CONCLUSIONS

The foliage surface limits for the Romanian varieties ranged between 1226 and 2558 $mm^2$ and for the foreign varieties between 1194 and 2785 $mm^2$.

Izvor variety, known for its capacity for drought resistence, registered highly significant decreases of the foliage area when compared to the Dropia witness and when compared to the average value, it registered significant decreases.

Nathan variety, in the first 4 hours of withering lost less water from the flag leaf than compared to others analised varieties, but registered a significant water loss in the next 20h.

Gruia variety registered a highly significant increase of water loss velocity in the first 4 hours and in the next 20 hours of withering the water loss was stabilised and registered a highly significant decrease.

Varieties that lost the most water in the first 4 hours after the leaves were excised lost less water in the the next 20 hours of withering.

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**Vol. XVII ( LIII ) - 2012**

# THE RELATIONSHIP BETWEEN YIELD AND PROTEIN CONTENT FOR AN ASSORTMENT OF WINTER WHEAT DURING 2008-2011 ON LUVOSOIL FROM SIMNIC AREA

Păunescu Gabriela[1], Olaru L.[2], Tuță Claudia[1]

***Keywords****: wheat, yield, content of protein, protein-yield relation*

## ABSTRACT

*During four years on luvosoil from Simnic have been evaluated 50 winter wheat varieties in terms of protein content and yielding capacity. Based on the relation between these two parameters were identified those varieties which emphasized simultaneously both yielding capacity and protein content up to the mean of tested varieties: Gruia (5469 kg/ha and 12,1% protein content), Glosa (5126 kg/ha and 11,8%), Delabrad (5130 kg/ha and 12,3%), Crina (510 kg/ha and 11,7%), Simnic 50 (5050 kg/ha and 12,3%), Ciprian (5020 kg/ha and 11,9%), Enesco (5516 kg/ha and 11,9%), Exotic (5158 kg/ha and 11,9%), GK Petur (5169 kg/ha and 11,7%), Mv Palma (5108 kg/ha and 12,1%), Cubus (5098 kg/ha and 11,9%), Frini (5899 kg/ha and 11,8%). Regression equation showed that for tested wheat assortment and climatically conditions during 2008-2011, for each increase of protein content with 1 % the yield decreases with 221 kg/ha.*

## INTRODUCTION

Grain protein content of wheat depends largely by variety, cropping technology (irrigated, rainfed, fertilizing) and natural soil conditions. Generally, high protein content is associated with good baking quality (Lăzureanu et al., 2009).

Among all technological factors, fertilizing influence highly grain protein content (Hera et al., 1986). Also among wheat technology elements, nitrogen fertilizers greatly influence the high level of grain protein content. Wet gluten content and falling number are favorably influenced by nitrogen fertilizer application (Naidin et al., 2000). Previous crop may also contribute to increased protein content. On these terms leguminous crops are important especially by their capacity to grow soil nitrogen content due to nitrogen fixing bacteria.

Following a successful pea crop soil nitrogen increases with 30-50 kg/ha (Burlacu et al., 2007). This aspect allows obtaining high protein yields per hectare using smaller amounts of fertilizers.

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Determination of protein content can be made by Kjeldahl method or different devices and analyzers.

Low-protein flour is suitable for snacks and cakes while high-protein flour is suitable for rough textured products like bread. Bakers use information about grain protein content to predict water absorption and dough development time during processing and production, known that high-protein flour require more water and longer time for mixing dough in order to gain optimal consistency.

## MATERIAL AND METHODS

During 2008-2011 in Simnic areas have been tested 50 Romanian and foreign winter wheat varieties in terms on protein content and yielding capacity.

The tested wheat varieties had different origins as follows:

Romanian varieties: Albota 69, Alex, Boema, Ciprian, Crina, Delabrad, Dor, Dropia, Faur, Flamura 85, Glosa, Gruia, Izvor, Lovrin 34, Romulus, Simnic 30, Simnic 50, Trivale.

Varieties from France: Apache, Autan, Aztec, Cezanne, Enesco, Exotic, Isengrain, Meunier, Renan.

Varieties from Austria and Germany: Capo, Cubus, Dunai, Fridoline, Josef.

Varieties from Hungary: Carolina, GK Elet, GK Miska, GK Petur, GK Othalom, GK Gobe, GK Kalasz, Mv Mariska, Mv Palma, Mv Magvas, Mv Marsall, Kiskun Serina.

Varieties from ex-soviet union: Bezostaia

Varieties from other UE countries: Bercy, Cordiale, Pobeda

Varieties from Serbia: Frini, Renesansa

Varieties from SUA: Orion

Protein content was determined using PERTEN INFRAMATIC analyzer. After cleaning impurities, wheat sample was introduced into the drum unit up to the mark and set for wheat program. Result is expressed in percentage and is on the device.

In terms of protein content, wheat grain is high quality to values up to 13%, good quality to values between 12-13%, satisfactory quality to values between 10-12% and poor quality to values below 10%.

A breeding strategy may be to identify those genotypes which deviate from the relation between protein content and yielding capacity proposed by Monoghan et al (2001) and Oury & Grodin (2007) which is used also in present study.

## RESULTS AND DISCUSSION

On three years average the lowest protein content was recorded by GK Miska (10,3%), while the highest value was recorded by Capo (13%).

The relationship yield-protein content enables to identify those varieties that have achieved both yield (4972 kg/ha) and protein content (11,7%) up to mean value. These varieties are placed in quadrant 3 of the graph shown in figure 1 and bring the best results in terms of both yield and quality.

The varieties with the best results in terms of relation yield-protein content were:

-Romanian varieties Gruia (5469 kg/ha and 12,1% protein content), Glosa (5126 kg/ha and 11,8%), Crina (5010 kg/ha and 11,7%), Simnic 50 (5050 kg/ha and 12,3%), and Ciprian (5020 kg/ha and 11,9);

-varieties from France: Enesco (5516 kg/ha), and Exotic (5158 kg/ha and 11,95);

-varieties from Hungary: Gk Petur (5169 kg/ha and 11,7%) and Mv Palma (5108 kg/ha and 12,1%);

- Austrian variety Cubus (5098 kg/ha and 11,9%);
- Serbian variety Frini (5899 kg/ha and 11,8%).

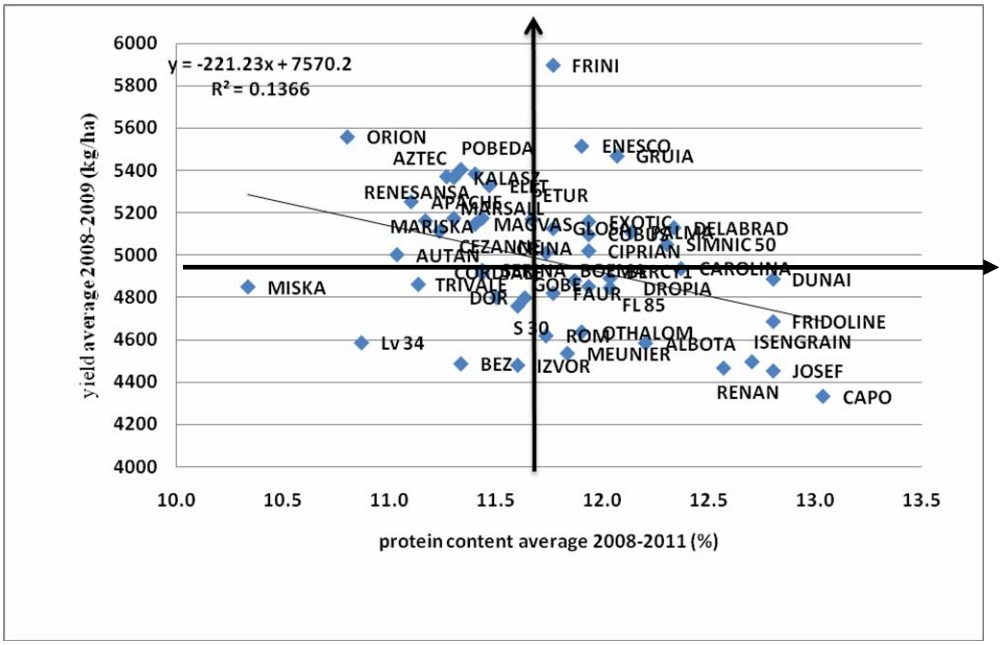


Fig.1. The relationship between yield-protein content for an assortment of winter wheat tested during 2008-2011

In terms of yields up to mean value of tested varieties but with low protein content were revealed the following varieties: Orion, Pobeda, Aztec, Kalasz, Renesansa, Elet, Apache, Marsall, Mariska, Magvas, Cezanne, Autan.

In terms of protein content up to mean of tested varieties but with low yield were revealed the following varieties: Carolina, Boema, Bercy, Dunai, Dropia, Gobe, Faur, Flamura 85, Fridoline, Othalom, Romulus, Albota, Meunier, Isengrain, Josef, Renan and Capo.

Varieties which didn`t meet any yield and quality up to mean value were less: Serina, Cordiale, Trivale, Dor, Simnic 30, Lovrin 34, Izvor, Bezostaia and Miska.

The varieties Delabrad, Capo and Joseph showed in the experimental conditions from NARDI Fundulea positive deviation from regression between protein content and two indices determined by Reomix devise, suggesting that these varieties are characterized by dough strength (Amalia Neacsu, 2012).

The regression equation shows that in the conditions of experimental area during 2008-2011, for each increase of protein content with 1% the yield value decreases with 221 kg/ha, the relation being negative as previous studies showed. Grain protein concentration

was negatively associated with grain yield especially when nitrogen fertilizer was applied (Marinciu Cristina & Săulescu, 2008).

This negative correlation between yield and protein content is undesirable in those varieties used especially for baking because normally protein content is positively correlated with bread volume (DePauw et al., 2007).

A previous study (Fossati et al., 2011) that included all wheat lines and varieties tested during 1987-2010 in officially and preliminary trials revealed a strong and negative correlation (r = -0,600).

## CONCLUSIONS

In the conditions from Simnic luvosoil during 2008-2011 the relation yield-protein content is negative one.

In those conditions the varieties with the best results in terms of relation yield-protein content were: Gruia, Glosa, Delabrad, Crina, Simnic 50, Ciprian, Enesco, Exotic, GK Petur, Mv Palma, Cubus and Frini.

The varieties which didn`t record good yield and quality up to average mean were: Serina, Cordiale, Trivale, Dor, Simnic 30, Lovrin, Izvor, Bezostaia and Miska.

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# YIELD AND QUALITY OF BAKERY AUTUMN WHEAT VARIETIES UNDER DIFFERENT TECHNOLOGY, FROM LUVISOIL FROM SIMNIC

Păunescu Gabriela[1], Rotaru A.[2], Tută Claudia[1], Păunescu Aida Ramona[1]

*Key words : wheat, yield, protein content, W value, different management technologic*

## ABSTRACT

*For three years (2009-2011), 25 autumn wheat varieties were tested in different technological conditions: the N16 P80 fertilized, fertilized with N100 P80, sown at normal and late seeding date. Yield increases were achieved by increasing nitrogen dose and product quality improved significantly by delaying sowing. Increasing the dose of nitrogen was reflected in yield increases, protein content and W value, all provided statistical. In the other hand, delaying sowing resulted in an increase in protein content, but also to reduce production, however statistically. From the evaluation results in four technological conditions revealed that Romanian varieties were best behaviour.*

## INTRODUCTION

Traditionally, improvement of common wheat is concentrated largely due importance to improve protein quality protein in bread, final product quality, nutritional value and economic impact (Suchy et al., 2007). To predict the quality of flour and dough numerous physical, chemical and rheological be determined (Gomez-Becerra et al., 2010).

There are many tests to determine the quality of wheat for making various bakery products. Among them, protein grain and flour ash content, falling number, flour color, dough rheology and sedimentation measured by farinograph, alveograph and / or mixograph are commonly used (Hruskova & Smejda, 2003, Rees et al., 2007).

Alveograph parameters important for bread volume, very significantly correlated with physico-chemical parameters of plain flour. Thus, a study of 100 grain flours derived from Romanian showed that the best predictors for alveografic W parameter were wet gluten index, falling number and protein content (Popa, 2007).

At A.R.D.S. Şimnic were tested 25 Romanian and foreign wheat cultivars in four technological conditions: autumn fertilization only P80 N16, N100 P80 fertilization applied in autumn and spring sown both in normal times and sown wheat growing period late. The data studied showed that value (W), a particularly important, can be predicted based on correlations between it and hectolitre mass and between it and the sedimentation rate,

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irrespective of the technology. When sowing is carried out at normal time, power is correlated with protein flour (Rotaru et al., 2010).

## MATERIAL AND METHOD

In 2009-2012 the S.C..D. A. Simnic were placed 2 bifactorial experiences, as follows:

Experience 1: Factor A: variety with 25 graduations Factor B: level of fertilization with 2 graduation N16P80 and N100P80

Experience 2: Factor A: variety with 25 graduations ( the same varieties of first experience) Factor B: seeding time with 25 graduations october 15-october 30

The varieties chosen were those that were approved at the beginning experiment Romania and entered in the official catalogue of varieties of crop plants in Romania 2007 edition: Dropia, Flamura 85, Boema, Glosa, Exotic, Alex, Apache, Bercy, Cezanne , Enesco, Elet, Gobe, Kalasz, Miska, Othalom, Petur, Serina, Magvas, Marsall, Palma, Pobeda, Renan, Renesansa, Gruia, Simnic 50.

The experiments were located in plots with 2 factors. Harvested area of each plot was 1.5 m wide and 6 m long.

Of statistical parameters that characterize the best variability were taken into account the arithmetic mean, standard deviation, and coefficient of variation limits of variability.

Characters were analyzed in terms of variability of: yield, protein content and W value.

## RESULTS AND DISCUSSIONS

Yield. Nitrogen and phosphorus are two of the most important nutrients in the world's grain yield (Calderini et al. 1995).

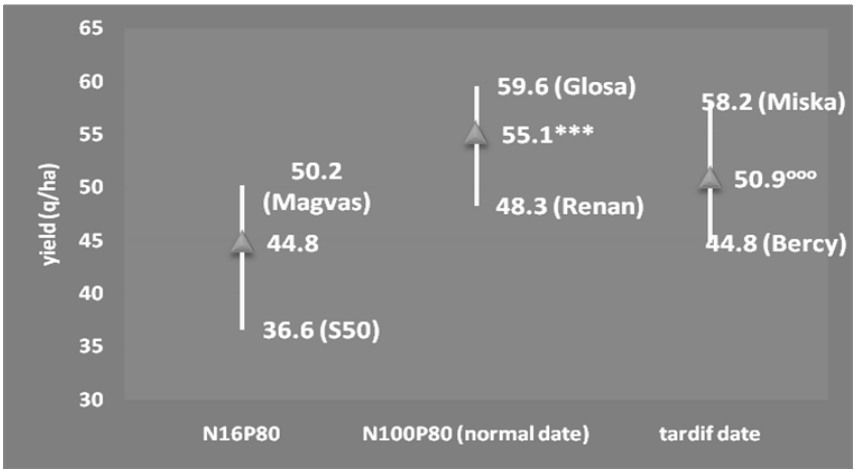
At Şimnic, under fertilization at spring, yield was very significantly higher versions when fertilization was conducted in the autumn of complex fertilizers, yiled growth was 10.3 q / ha.

In a study conducted during 2005-2009 ARDS Podu Iloaie three doses of fertilizer (without nitrogen, N60 and N120) P80K80 background of the last two and a growing assortment of 12 Romanian wheat production was highest obtained from alternative high dose of nitrogen, as in our study, the variety Faur (Cojocaru & Carmen Ghita).

Late sowing resulted in very significant reduction in production. Intersecting values are only a short interval from 48.3 to 50.2 kg / ha, which shows a sharp delineation of the character values according to experimental conditions, especially between levels of fertilization (Figure 1). Same result - reducing yield with delayed sowing were obtained in other studies in which sowing was delayed two weeks (Sulek 2009, Rotaru et al., 2010).

Maximum and minimum values are recorded for different varieties, which indicates that the tested varieties differentiated by technology potential applied.

Intersection values for different levels of fertilization takes place on a single range (48-52 q / ha), while for different planting dates are two classes of common stock (48-52 q / ha and 52 q / ha) .




NEF/FERT DL 5% = 2.6 q/ha; DL 1% = 3.5 q/ha; DL 0.1% = 4.5 q/ha
NOR/TARD DL 5% = 2.4 q/ha; DL 1% = 3.2 q/ha; DL 0.1% = 4.2 q/ha

Fig. 1. Maximum, minimum and average production varieties tested in different technological conditions (average 2009-2011).

From the graphical representation of fertilizer production in both conditions showed that varieties had yields above average varieties tested were: Cezanne, Renessansa, Pobeda, Apache, Marsall, Elet and Alex (Figure 2).

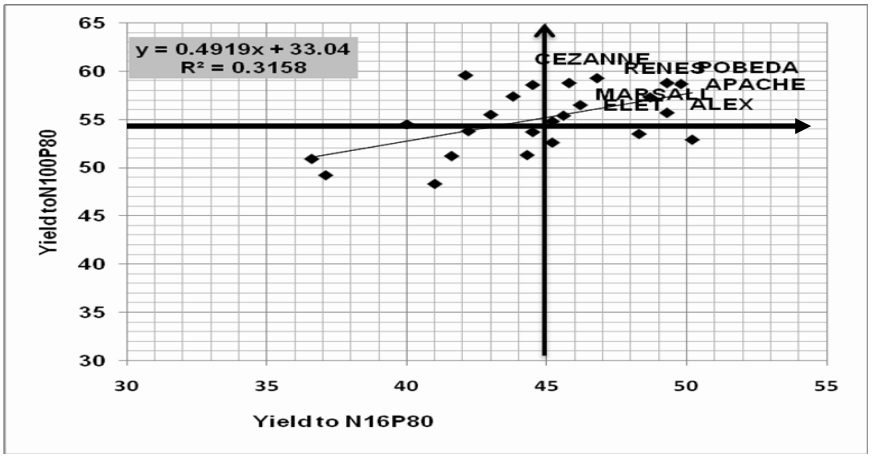



Fig.2.Varieties with above average yield in both conditions of fertilization

Depending on planting dates were noted with above average yields at both planting dates: Miska, Renaissance, Gruia, Marshall, Apache, Cezanne, Enesco, Glos, Elet, Kalasz, Petur (Figure 3).

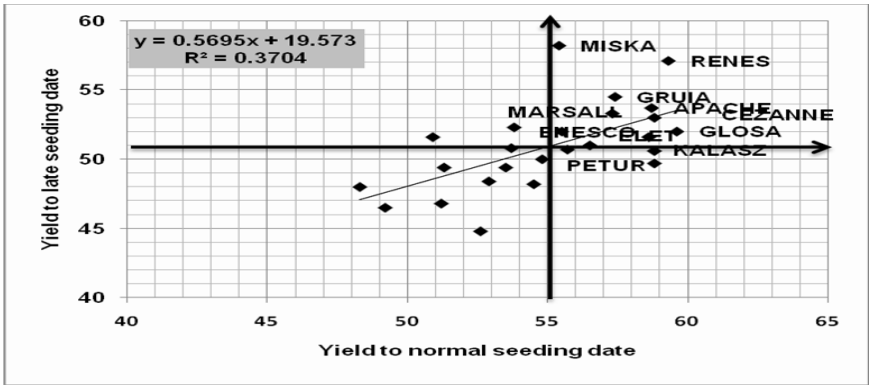
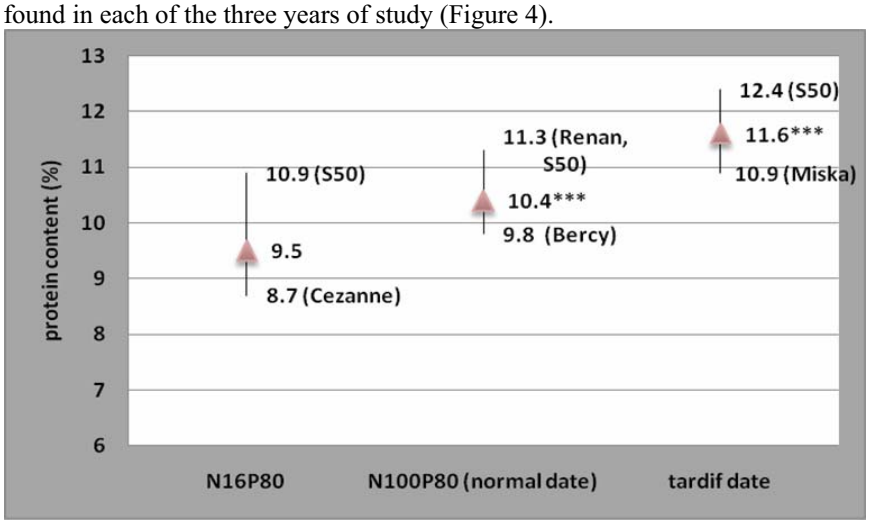



Fig.3 Varieties with above average yield in both seeding dates

**Protein content**. Is most affected indicator of the study.

An increase in the dose of nitrogen is observed a very significant increase protein content of 0.9%, but it should be noted that the average values which do not reach the download limit is 10.5%.

At a delay of two weeks sowing recorded a growth of 1.2% protein content. This was found in each of the three years of study (Figure 4).




| | |
|---|---|
| NEF/FERT | DL 5% = 0.4 %; DL 1% = 0.5 %; DL 0.1% = 0.7 % |
| NOR/TARD | DL 5% = 0.4 %; DL 1% = 0.6 %; DL 0.1% = 0.7 % |

Fig. 4. Maximum, minimum and average protein content in different technological conditions (average 3 years)

Same result - increased protein content with delayed sowing were obtained in other studies in which sowing was delayed two weeks (Sulek 2009, Rotaru et al., 2010).

We suggest to the results, a simple delay in sowing in soil and climatic conditions of the area indices Şimnic result in much improved baking quality, obviously if it seeks only that.

Specifically noted the variety Şimnic 50 which in all experimental conditions to obtain the highest protein content over the download limit.

The minimum values were recorded foreign wheat varieties.

Shared between three technological conditions is practically confined to a single value - 10.9%, which shows a strict separation and concentration of graduation results by factor B - fertilization level and time of sowing.

Under different conditions of fertilization, varieties react strongly to increasing the dose of nitrogen, the coefficient of determination was 66%. Varieties with above average protein content at both doses were: Renan, Simnic 50, Dropia, Banner 85, Renaissance, Exotic, Boema , Palma, Gruia, Glosa, Enesco. In addition, we mentioned that the varieties româneşri Şimnic 50, Banner Drop and 85 are the only ones that were more than 10.5% protein (download limit) fertilization in both conditions ( Figure 5).

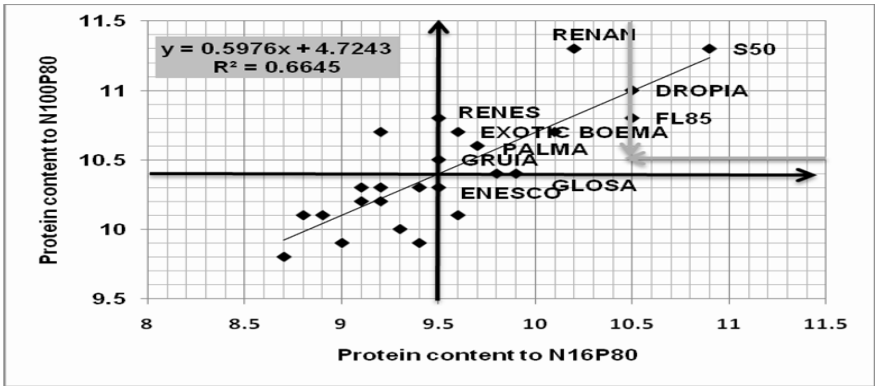



Fig. 5 Varieties with above average protein content in both conditions of fertilization

Regardless of planting dates, varieties Şimnic 50, Renan, Exotic, Palma, Banner 85, Dropia, Boema had a protein content higher than the average for all varieties tested. Beyond the acquisition of 10.5% of the above varieties are added Gruia, Marsall, Renaissance.

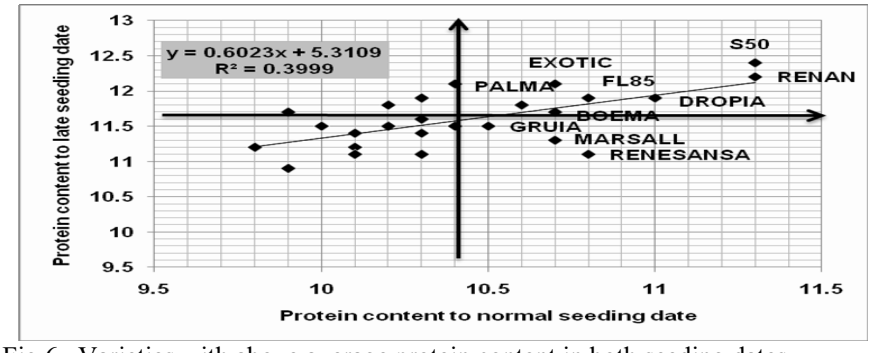


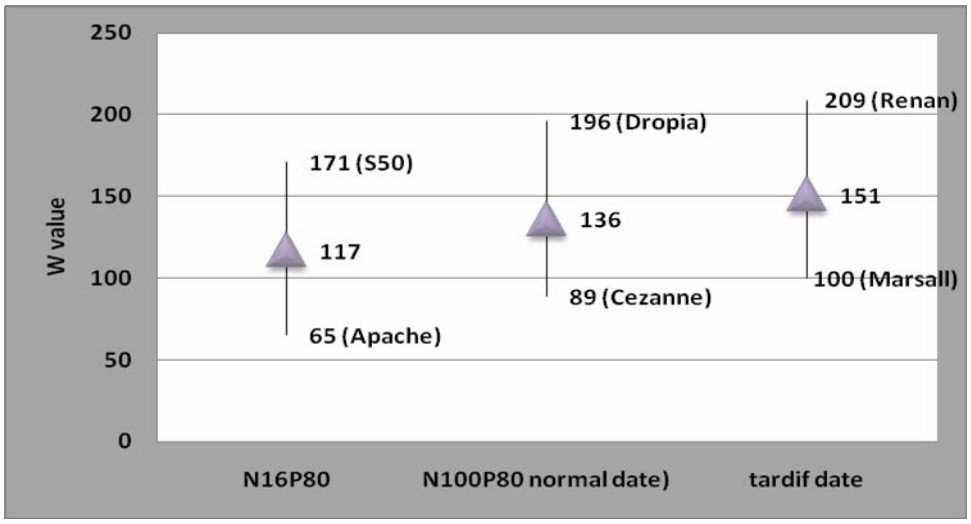

Fig.6 . Varieties with above average protein content in both seeding dates

**W Value.** A distinctly significant power increase was recorded only when flour was increased dose of nitrogen (from 115 joules to 151) ( Figure 7).

High values were recorded only in three of Romanian varieties that technological conditions showed values above 200 joules, as required qualitative assessment protocol.

Seeding did not affect the indicator studied, only 8 joules increase the normal time period is not statistically assured late.

Above average varieties tested, regardless of fertilization ranged varieties: Dropia Flamura 85, Glosa, Renan, Boema , Kalasz, Gruia, Pobeda, Palma ( Figure 8).




NEF/FERT DL 5% = 23 jouli; DL 1% = 31 jouli ; DL 0.1% = 40 jouli

NOR/TARD DL 5% = 29 jouli; DL 1% = 40 jouli; DL 0.1% = 52 jouli

Fig. 7. Maximum, minimum and average power under different technological flour (average 2009-2011)

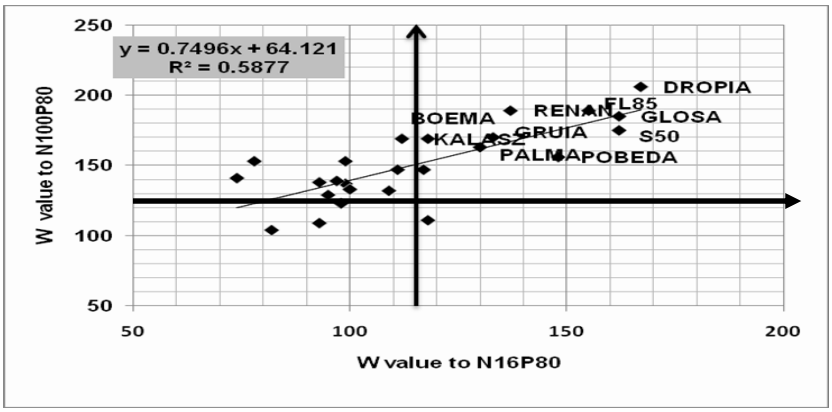



Fig .8. Wheat varieties with W value above average in both fertility conditions

Above average varieties, regardless of the seeding, in terms of value varieties were ranked: Flamura 85, Dropia, Şimnic 50, Boema, Renan, Glosa, Kalasz, Magvas. Above the 200, both at the normal time and the late time, was given only variety Dropia (Image 9).

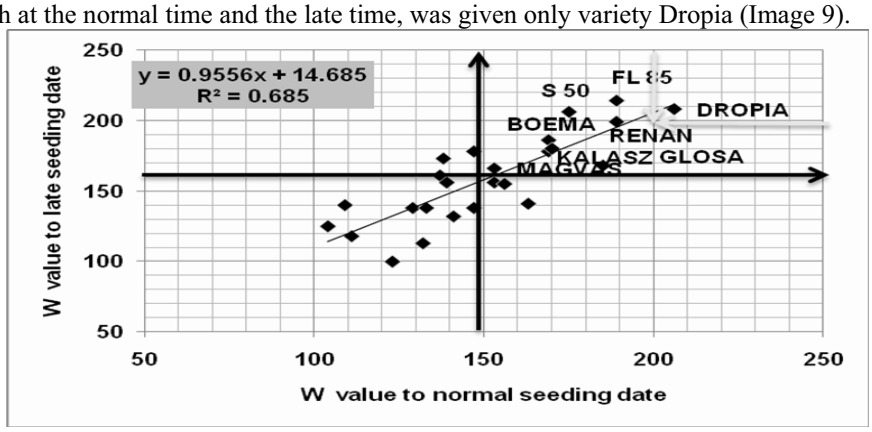



Fig .9 Wheat varieties with W value above average in both seeding dates

## CONCLUSIONS

Production increases were achieved by increasing nitrogen dose and product quality improved significantly by delaying sowing.

Increasing the dose of nitrogen was reflected in increases in yield, protein content and value, however statistically.

On the other hand, delaying sowing resulted in an increase in protein content, but also to reduce production, however statistically.

From the evaluation results in four technological conditions revealed that Romanian varieties were best behaviour.

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**Vol. XVII ( LIII ) - 2012**

# FILOGENETIC RELATIONSHIPS AMONG *ALLIUM SATIVUM* L. LANDRACES BASED ON DNA MARKERS

Petcov Andreea Adriana [1], Corneanu Mihaela [1],

***Keywords****: landraces, variability, Allium sativum L.*

## ABSTRACT

For the molecular tests, there have been used fresh biological stuff, that were the leafes colected from the tested landraces coming from 16 places of local allium sativum L After the DNA extraction from allium sativum leafes, the quality and quantity of it, were checked using the spectrofotometric method and the migration in agarosis gel, after being colored in ethidium brom and viewed on UV light. In order to have a objective results for the electroforegrams, there have been calculated the polimorfism (P, %) for the amplified parts of DNA. For the garlic landraces the genetic polimorfism that was obtained is 16,39%. The geografic distances beetwen the areas that were colected the garlics are not very large, that why the polimorfism is low. Also it have been noticed that generally, the stability of the garlic genom is high. The bands number obtained after the amplification had the variation 2 (Primer RAPD 2) and 10 (Primer RAPD 5). The intensity of the bands was also different, there have been a low intensity bands, medium or high ones. Based on the number of the amplified bands there have been calculated the genetic distances betwen the landraces. By the UPGMA filogenetic clasification of the analized genotipes and from the obtained cluster, it shows a very close grouping by the areas that the landraces are coming from.

## INTRODUCTION

Garlic cultivation (*Allium sativum* L.) is so old as the human race and so wide as the civilization itself. References for this plant can be found in the Bible and Coran, reflecting the importance as a food and as a horticulture plant (**Tapsell et al**, **2006)**.

*Alliaceae* family comprises one of the most important plant groups, considering the economic advantages, like garlic (*Allium sativum* L.), onion (*Allium cepa*L.) **(**Brewster, 1994).

Garlic is rich in antioxidants that prevent formation of free radicals -particles that damage celular membrane, DNA and have a major contribution to aging and disease development (Rivlin, 2006,2001). Genetic diversity is a fundamental component of biodiversity as it is the basis of species diversity and ecosystem diversity. Genetic diversity

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can be characterized by the set of possible alleles (different variants of the same gene) and their frequencies, by entire genes, or by even units larger than genes such as structures on chromosomes (Nei, 1987; Lowe et al. 2004).

## MATERIALS AND METHODS

**Biological material.**

Biological material consisted from 16 garlic landraces (*Allium sativum* L.) collected from Timis, Arad and Hunedoara County (table 1).

Table 1. General characteristics of *Allium sativum* landraces at *in situ* collection time

| No. | Catalog No. | Collection site/Address | Observations |
|---|---|---|---|
| 1 | 1252 | Mărăuş, nr. 18, jud. AR | autumn garlic |
| 2 | 1269 | Şeitin, jud. AR | autumn garlic |
| 3 | 1484 | Căpâlnaş, jud. AR | autumn garlic |
| 4 | 1763 | Sebiş nr. 5, jud AR | spring garlic |
| 5 | 1765 | Sălăjeni nr. 21, jud. AR | spring garlic |
| 6 | 1768 | Sebiş, nr. 18, jud AR | spring garlic |
| 7 | 1770 | Sebiş, nr 94, jud AR | spring garlic |
| 8 | 1231 | Cenad,nr.1259, jud TM | spring garlic |
| 9 | 1235 | Chizătău, nr. 80, jud TM | spring garlic |
| 10 | 1279 | Căpăt, nr. 107, jud. TM | spring garlic |
| 11 | 1480 | Valcani, nr. 556, jud TM | spring garlic |
| 12 | 753 | Curechiu, nr. 95, jud TM | spring garlic |
| 13 | 750 | Băcâia, nr. 75, jud.HD | spring garlic |
| 14 | 754 | Poiana, nr 6, jud. HD | spring garlic |
| 15 | 755 | Poienița, NR 46, jud. HD | spring garlic |
| 16 | 772 | Oprişeşti, nr. 4, jud.HD | spring garlic |

Garlic landraces have been collected from original sites and all data regarding life cycle, yield, quality and other features have been registered in data sheets for each population. DNA have been extracted from 50 mg fresh leaves using 2xCTAB method (Saghai-Maroof, 1984, with few modifications). DNA was cleaned up DNA Clean Kit de ZymoResearch (BioZyme). For each PCR reaction 50 ng/µl was used in the mixture.

For RAPD reaction 10 oligomers OP-A (Operon Techologies, Aalmeda) were used (Table 2).

In case of polymorphism analysis we counted only those amplification bands that were clear and present in both repetitions.

For a correct quantification the amplified products were scored with 1 (for present) and with 0 (for absent). According to electrophoresis profiles a cluster could be constructed that indicated the similarity between garlic landraces. In this sense we made used of the available indices:

- Genetic similarity coefficient
- Genetic distance (DG) calculated as follows as a Jaccard coefficient:

$$J(i,j)=C_{ij}/(n_i+n_j)-C_{ij}$$

Using the - UPGMA (Unweighted Pairwise Group Method with Arithmetic Mean) similarity cluster has been assembled.

Table 2 RAPD oligomers sequence (Biosearch Technologies, INC)

| Reference No. | 5'-3' |
|---|---|
| 1 | P2-5'd(GGT-GGC-CAA-G)3' |
| 2 | P5-5'd(CAC-TGG-CCC-A)3' |
| 3 | P7- 5'd(TGG-TCG-GGT-G)3' |
| 4 | P8- 5'd(CTA-AGC-GCA)3' |
| 5 | P9- 5'd(TTG-CTG-GGC-G)3' |
| 6 | P11- 5'd(CCG-CTG-GAG-C)3' |
| 7 | P12 -5'd(CGG-AGA-GCG-A)3' |
| 8 | P13- 5'd(CGA-CCA-GAG-C)3' |
| 9 | P15- 5'd(GCT-CCC-CCA-C)3' |
| 10 | P16 -5'd(TTG-CTG-GGC-G)3' |

## RESULTS AND DISCUSSIONS

Molecular genetics has already had a tremendous impacts on plant breeding. Progress in the development of new PCR- based marker systems has opened the vast majority of plant genomes to investigation.( **David H. et al, 1999**). In the next figure (Fig.1) is presented the quality of DNA extracted from landraces of *Allium sativum* L.

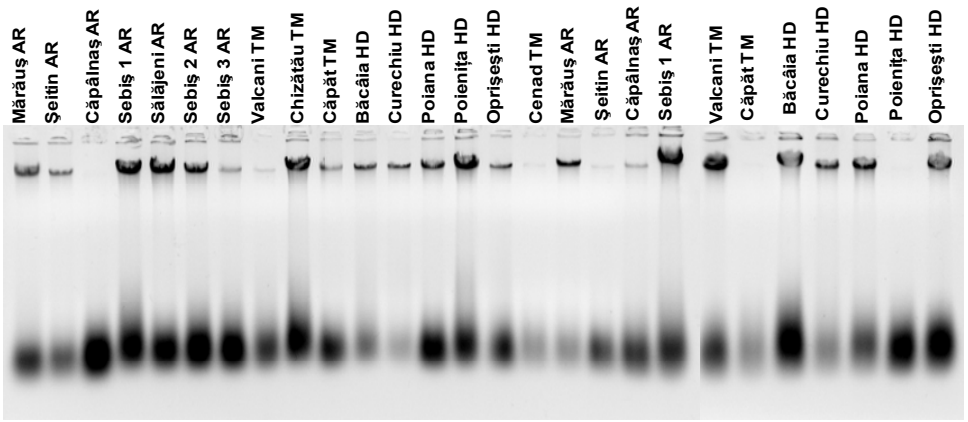


Fig. 1. DNA quality in garlic landraces by agarose gel (1%) electrophoresis. (1-16 single probes from each garlic landraces; 17-27 duplication of samples)

The figures below show the RAPD PCR amplification using different random oligomers in all 16 garlic populations (The Cenad garlic was used as control). The

amplification products were separated on agarose gel 1,2% and visualized after etidium bromide staining.

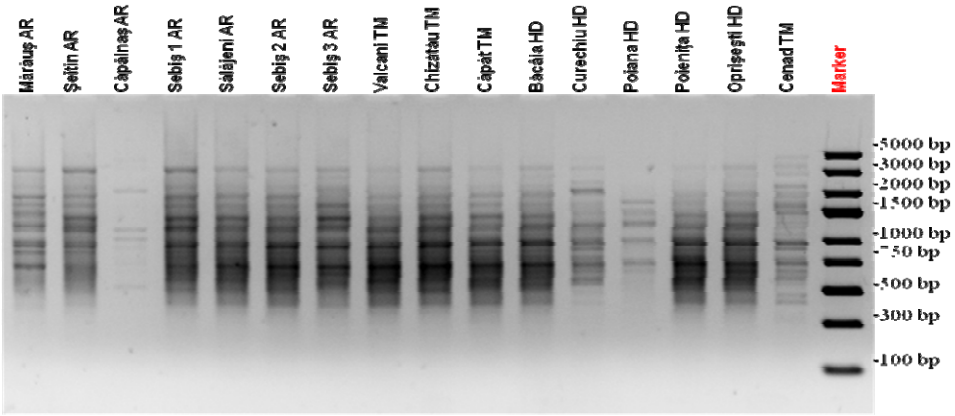


Fig. 2. RAPD profile in garlic landraces using oligomer P5-5'd(CAC-TGG-CCC-A)3'

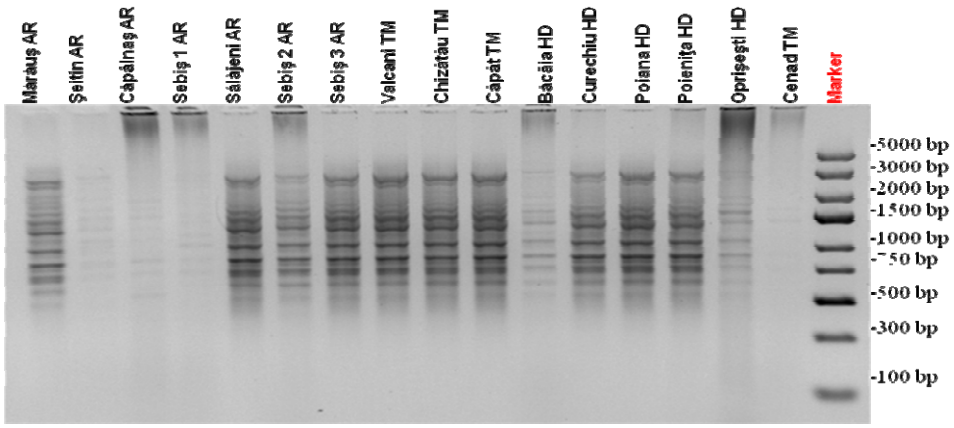


**Fig. 3. RAPD profile in garlic landraces using oligomer P9- 5'd(TTG-CTG-GGC-G)3'**

In case of RAPD (Fig. 2; 3), amplification the reproducibility of reaction is very important in order to validate the data. In this sense we repeated the DNA extraction and RAPD reaction from 2 independent samples and compared the bands for each reaction. In order to analyze the molecular polymorphism in garlic landraces we considered only that amplification band that was present in both repetitions. The polymorphism (P, %) was calculated according to presence and absence of bands compared to the total amplified bands in for each primer used. To appreciate the P of a population we considered that a single individual band is present in 94% of individuals from the group then the band is polymorphic. If a amplification band is present only in 6% of individuals that form the analyzed group the band is absent so it dose not appear in all other 94% of individuals so its polymorphic. If a band appears to 95% of the individuals then is a monomorphic. For a

better quantification the band were scored 1 for present and 0 for absent. Using the electrophoresis profiles we could build a cluster that reflects the similarity degree between garlic populations. The genetic distances were calculated using the Jaccard coefficient and UPGMA for dendogram construction.

In garlic landraces the polymorphism is low, 16.39% that can be explained by genetic stability of garlic and the close geographical origin of the collected landraces. The nomber of amplified bands varied form 2 (oligomer 2) to 10 (oligomer 5). The bands intensity can be classified as low, medium and intense. We can conclude that the garlic landraces are genetically stable and are closely related to each other.

The calculated genetic distances are below 0.5 which indicated a very close relation between the analyzed population and a high degree of similarity. The UPGMA clustering (Fig. 4) is grouping the garlic landraces into geographical associations and confirms the Cenad garlic as the oldest in the group. Few population are independently classified which indicates a separate evolution. (Uzun A., 2011)

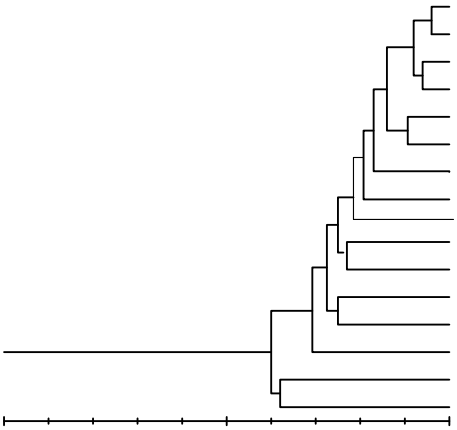

Fig. 4. Clustering of garlic landraces based on UPGMA analysis

There is a differentiation inside the geographical group. In all garlic landraces can see grouping bade on site of origin which indicated isolation based on distance model. Overall the genetic variability among analyzed landraces is low which indicated a conserved stable genome and behavior.

DNA markers also have been used extensively to characterize germplasm, a process popularly known as fingerprinting, to evaluate the genetic relationships among accessions (genetic diversity) and provide important information in the areas of ecology, population genetics, and evolution. (David H. et al, 1999)

Since we don't know how this landraces have appeared we can't conclude about their genetic base in our limited conditions for molecular characterization namely the RAPD technique. Other more sensitive markers will be able to discriminate better this landraces.

## CONCLUSIONS

1. Our results confirms necessity to analyze and select of new germplasm for improving garlic yield;
2. The biogenetic analysis based on DNA markers indicated very close relationships between garlic landraces and a high degree of similarity.
3. Beetwen the landraces that were analyzed, the genetic distances have the value under 0,50, that means a very close group and also a relative relation. The UPGMA test shows it, and also does a filogenetic clasification of the tested genotypes. The control genotype (Cenad), is making the cluster and the Seitin landrace is branching independent from the other genotypes, that shows a separate evolution from a common ancestor. From the obtained cluster it shows a very close group by the areas that landraces are coming from.

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# THE MONITORING OF AIR POLLUTION FROM AN URBAN AREA IN CRAIOVA CITY

Popescu Simona Mariana[1], Gavrilescu Elena

*Key words: urban pollution, traffic, industry, intersection, Craiova City.*

## ABSTRACT

*In the last years, the rapid industrialization, urbanization and traffic increase caused changes in the atmosphere by increasing the concentration of primary and secondary pollutants.The study was carried out in the year 2011, in an intersection from Craiova City, an area with a high traffic flow but also situated under the incidence of the pollutant emissions originating from the thermal power plant CET I Işalnița and consisted in the monitoring of four air pollutants: $O_3$, $SO_2$, $NO_2$, and $PM_{10}$. The monthly concentrations of the air pollutants monitored are within the limits imposed by the Law 104/2011, exceptions being observed for ozone, exceeding the limit in July and for particulate matter exceeding the limit in January, February March, October, November and December. Further studies including calculations of the dispersion of pollutants and their correlation with climatic factors and orography are necessary for a better understanding of air pollution.*

## INTRODUCTION

In the last years, the rapid industrialization, urbanization and traffic increase have caused changes in the atmosphere by increasing the concentration of primary and secondary pollutants.

The primary pollutants are emitted directly into the atmosphere from various stationary sources (industry) or mobile (traffic), whilst secondary pollutants are produced in the atmosphere from chemical reactions that occur between primary pollutants and various components of the atmosphere or even between primary pollutants.

All combustion releases gases and particles into the air. These can include sulphur and nitrogen oxides, carbon monoxide and soot particles, as well as smaller quantities of toxic metals, organic molecules and radioactive isotopes (Agbaire & Esiefarienrhe, 2009).

An "air pollutant" can be defined as any substance emitted into the air from an anthropogenic, biogenic, or geogenic source, that is either not part of the natural

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atmosphere or is present in higher concentrations than the natural atmosphere, and may cause a short-term or long-term adverse effect (Daly & Zannetti, 2007).

Air pollution can affect human health in many ways by affecting the lungs and the respiratory system, cardiovascular disease, as well as it can affect plants by processes such as stomatal closure, plants being unable to do gas exchange with the environment.

Global climate changes and air quality are closely coupled through many atmospheric meteorological, dynamic, chemical, and radiative processes (Karamchandani et. al. 2012).

Air pollutants are transported regionally over scales from about 100 to a few 1000s of kilometers, large enough to cross state, provincial, national, and even continental boundaries (Bergin et al. 2005), in this study being monitored four air pollutants, namely $O_3$, $SO_2$, $NO_2$, and $PM_{10}$.

$NO_x$ are air pollutants, and they are one of the precursors of photochemical reaction. Nearly 70% $SO_2$ in the atmosphere originates from fossil fuel combustion (Yang & Liu, 2011), whilst the main sources that have been identified for particulate matter are: thermo-electric power stations that use solid or liquid fuels, the house heating systems, constructions, mining, cement industry (Braghină et al. 2010).

Ozone ($O_3$) is a trace gas species resulting, in the troposphere, from the oxidation of carbonaceous species (CO, hydrocarbons) in the presence of NOx both emitted by anthropogenic and natural processes (Szopa et al. 2006). This ground-level ozone called tropospheric ozone is toxic and harmful to the environment.

Therefore this study aims at highlighting the level of atmospheric pollution from a high-traffic intersection of Craiova City to help identify the pollution sources and making the best decisions for the best management of air quality.

## MATERIALS AND METHOD

Craiova city is located in the centre of Oltenia on the left side of Jiu River, more precisely at 44°19'16" N, 23°48'33" E and at an altitude of 75-11m .

The climate of Craiova city is temperate continental, with annual average temperature of 10.7° C, for July and August the average temperature reaching 22,2 $^0$C, respectively 21.7 $^0$C and the annual average rainfall being of 569.9 mm, the most rainy months being June and May, with monthly average of 70.2 mm and respectively 63.4 mm (Sandu et al., 2008).

The major sources of air pollution in the city of Craiova are represented by anthropogenic activities which can be grouped in 3 categories: traffic, with specific pollutants emission from the combustion of fuels; various industrial activities with pollutant emissions like, gases, vapors or particulate matter - specific to different technological processes; specific urban activities with emissions mainly due to fossil fuel combustion.

The study was carried out in the year 2011, in an intersection from Craiova City, an area with a high traffic flow but also situated under the incidence of the pollutant emissions originating from the thermal power plant CET I Işalnița and consisted in the monitoring of four air pollutants: $O_3$, $SO_2$, $NO_2$, and $PM_{10}$.

The measurements were performed monthly using a portable gas detector Oldham (Model MX 21 – Plus Multigas, France) equipped with electrochemical sensors and which can measure up to 4 gases simultaneously, in this study being presented the monthly average of the concentrations measured for each gas separately.

## RESULTS AND DISCUSSIONS

Along with population growth and the development of small and medium enterprises as well as the continued use of polluting technologies there have been changes in climatic factors associated with the increase of pollutants in the atmosphere.

From the monitoring of air pollutants, made in 2011, in the intersection from Billa area in Craiova City the following observations were made:

1. The data obtained from monitoring nitrogen dioxide in the studied urban area highlighted that $NO_2$ is within the limit provided in the Law 104/2011, below 200 µg/m$^3$. The intensification of traffic sometimes led to an increase of the nitrogen dioxide concentration up to 48,42 µg/m$^3$, in August, without exceeding the limit. It can be seen from Fig. 1, that nitrogen dioxide recorded higher values during the summer months, due to increased road traffic being emitted in the air large amounts of nitrogen monoxide which is oxidized to nitrogen dioxide in the presence of solar radiation.

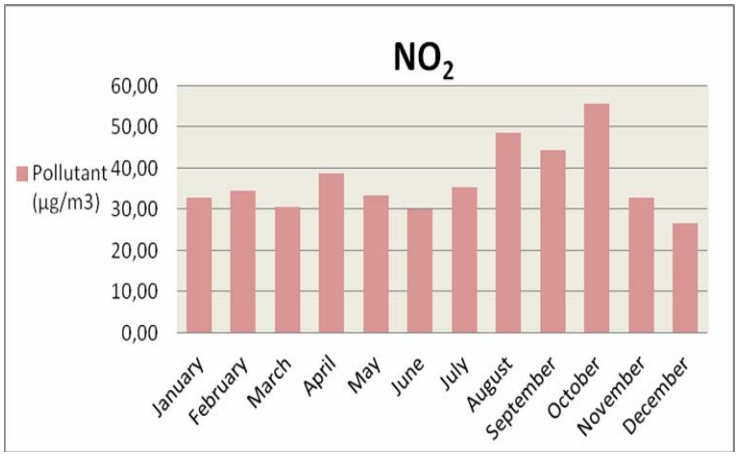


Figure 1. The nitrogen dioxide monitoring during the year of 2011 in Craiova City

2. Sulfur dioxide is emitted in the atmosphere mainly from combustion processes of fossil fuels, which have a higher sulfur content, processes that occur in our area from power plants and domestic heating. In the case of sulfur dioxide there were observed higher values during the summer months, with a maximum of 44,10 µg/m$^3$ observed in August (Fig. 2), but not exceeding the permissible limit of 125 µg/m$^3$ required by the Law 104/2011. Once the operations of improving the desulphurization installations is completed, unfortunately delayed from various technical and financial reasons, it is expected to lead to lower growth events of $SO_2$ concentrations in the atmosphere.

3. The highest values for the particulate matter monitored (Fig. 3) occurred during the winter months during which their emission sources are amplified mainly due to the need of house heating. Most of the exceedances occured in January (65,42 µg/m$^3$), February (56, 00 µg/m$^3$), March (79,32 µg/m$^3$), Octomber (57,65 µg/m$^3$), November (63,25 µg/m$^3$) and December (90,55 µg/m$^3$). The continuation of the traffic fluidization projects in the city, the improvement of slag and ash disposal systems and of particulate matter retention

systems at the 2 power plants in the city, as well as the continuation of the maintenance and increase green areas politics in the urban area of the city and surroundings can make a major contribution in achieving lower concentrations of $P_{M10}$ in the future.

4. Ozone monthly averages recorded for the year 2011 showed a minimum value of 36,45µg/$m^3$ in January, its highest values being observed in June, July, August and

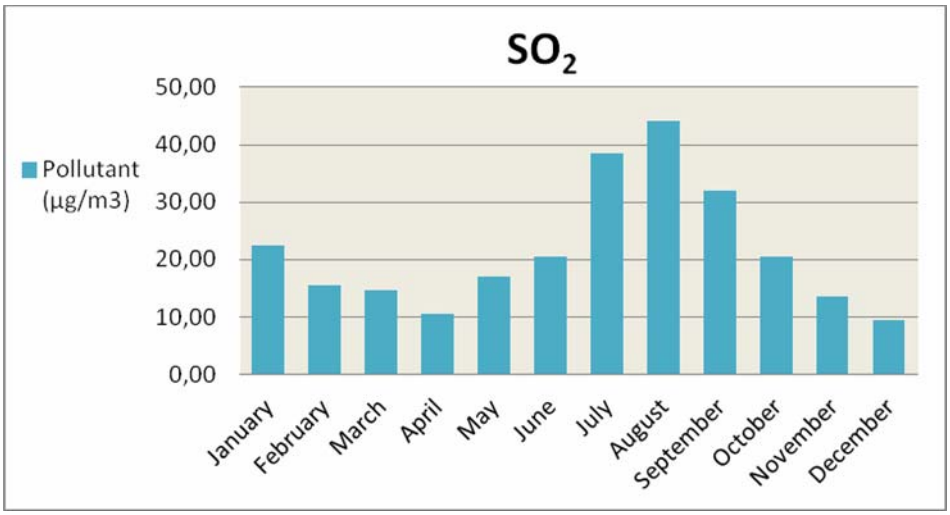


Figure 2. The sulfur dioxide monitoring during the year of 2011 in Craiova City

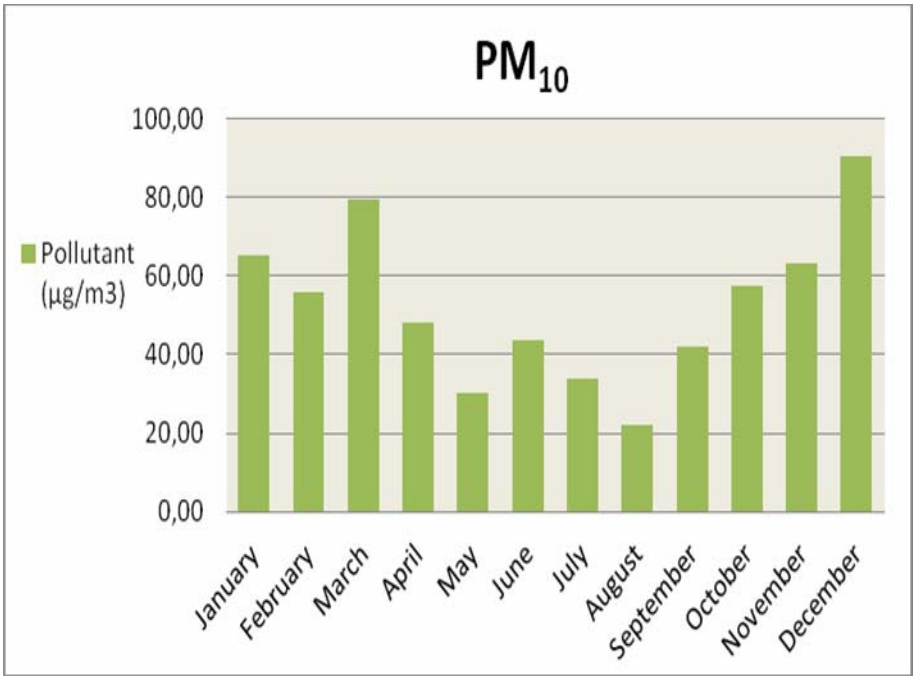


Figure 3. The particulate matter monitoring during the year of 2011 in Craiova City

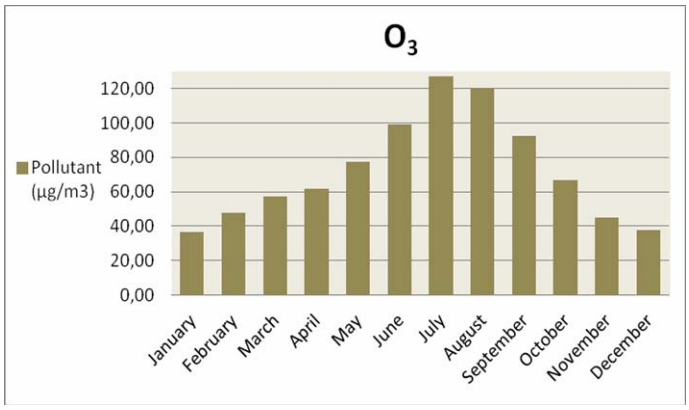

Figure 4. The ozone monitoring during the year of 2011 in Craiova City

September, stating that the value observed in July, of 127,00 µg/m$^3$ exceeded the maximum allowed according to the Law 104/2011, respectively 125 µg/m$^3$ as it can be seen in the Fig. 4. Exceeding the limit value has occurred, probably due to increased road traffic and accordingly to the emission in the atmosphere of ozone precursors, to which were added favorable climatic conditions for ozone production, namely high temperatures and solar radiation.

Similar studies regarding the monitoring of air pollutants associated with emissions from road traffic were also made by Brauer et al. 2002, Kim et al. 2004.

## CONCLUSIONS

From the data obtained after monitoring the quality of the environment in the Billa area from Craiova Cit, the study highlights that:

Nitrogen dioxide recorded higher values during the summer months, not exceeding the maximum permitted by the Law 104/2011.

Monthly monitoring of sulfur dioxide indicated a higher value in august of 2011. As in the case of nitrogen dioxide, $SO_2$ also recorded higher values during the summer months, but not exeeceding the limit.

In the case of particulate matter there have been observed exceedances of the maximum permitted during the winter months, the $P_{M10}$ source being road traffic and also the industry from the city.

The ozone recorded oscillating values, being observed an exceeding of the permitted limit in July of the year 2011, due to more favorable conditions for its formation in the troposphere. In the case of ozone, the formation of which is highly dependent on weather conditions and of the conditions of its precursor concentrations in the atmosphere at high levels, very favorable to its formation in the city of Craiova during April-September, reducing its concentration is very difficult to achieve.

This study complements only part of the problem complexity, further studies including calculations of the dispersion of pollutants and their correlation with climatic factors and orography being necessary for a better understanding in how air pollutants can affect the environment.

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# BIOMEDICAL COMPOUNDS IDENTIFIED IN *CIRSIUM ARVENSE* BY GC-MS

Radu Mihaela[1], Chirigiu L.[2], Bubulică Maria-Viorica[2*]

***Key words*:** *Cirsium Arvense, GC-MS, pharmaceutical compounds*

## ABSTRACT

*The study was made by gas-chromatography coupled with mass spectroscopy on ethanolic extract of one of most popular weed from our country, thistle. After GC-MS analyses have been identified in this plant a great number of polyphenols and flavonols with uses in medicine and phytopharmacy. Among the identified compounds the ones that have pharmaceutical importance are: quercetin-3-O-glucoside, quercetin-3-O-rutinoside, ferulic acid, kaempferol-3-O-rutinoside and 3,4-dimetoxy cinnamic acid.*

## INTRODUCTION

*Cirsium arvense* (thistle) is a common weed with roots deeply embedded in the earth that can reach to 1.5 - 2 m, with pink purple flowers 1-2 cm in diameter. Thistle needs light, nutrients and water in higher proportions than other plants (Pop et al., 1983).

*Cirsium arvense* is a considerable problem for many farms in the soil culture and it grows especially on good land. But, following basic principles (rotations, soil work), this weed can be controlled.

*Qualities and curative medical action*

So far thistle is mentioned in some ethno-therapeutic recipes where the roots are used in local baths to treat hemorrhoids and varicose veins.

For this purpose it is used the entire plant, with roots, washed well and chopped. Boil for 1 hour and and let the bowl covered 4 hours. Feet are packed with thistle broth in this way: soak unpainted strips of cotton or linen and applied them on the legs from top to the bottom. Wrapped legs are held for 4 hours.

No other organ of this plant is mentioned.

Our research aims to analyze the chemical composition of plants from indigenous flora, especially those that are widespread and not investigated until now.

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Project results follow introduction into medicinal plant category, of those plants unused, known as weeds and fiercely controlled by farmers. If it turns out that in their composition are biologically active compounds, because of their abundance, they will represent for the pharmaceutical industry an inexhaustible and convenient source of raw materials, and for physiotherapy, a huge source of medicines available to all.

## MATERIALS AND METHODS

*Plant material*

*Cirsium arvense* have been collected from Craiova, Dolj County, Romania, in August 2010. The plant was dried at temperatures comprised between 20-22$^{o}$C, in a dark room, at constant humidity (60%).

*Sample extraction method*

3 g of the finely ground plant were weighed, over which 30 mL of ethanol were added. The sample was maintained on a water bath at a 50$^{o}$C temperature for 8 hours, it was filtered and analyzed in GC-MS.

*GC-MS analysis*

The sample of *Lonicera tatarica* leaves was analyzed in:

- gas chromatography coupled with mass spectrometry (Agilent 7890 A GC System – 5975C VL-MSD)

*Chromatographic conditions*

Non-polar column Agilent 1909 s-433 (5%-phenyl methyl siloxane)

Carrier gas: He, flow 1ml/min. Oven temperature was set at 70$^{o}$C for 2 minutes, then it was programmed for a 270$^{o}$C temperature with a rate of 20$^{o}$C/min. The injector temperature was 280$^{o}$C. Injection mode: splitless. Injected volume: 4 µl. The operating parameters of the mass spectrometer: ionization potential 70eV.

Interface temperature: 200$^{o}$C; acquisition of spectres was between 50-800 m/z. For identifications, the NIST 05 mass spectre/structures library was used.

## RESULTS AND DISCUSSION

The identified compounds of Cirsium arvense leaves and stems are presented in table 1. The obtained chromatograms for *C. arvense are* shown in figure 1 and figure 2.

*Bio-medical considerations*

Among the identified compounds in gas chromatography coupled with mass spectrometry, the ones that have pharmaceutical importance are:

- *quercetin-3-O-glucoside* (Q3G) has cytotoxic, phytotoxic, antimicrobial and antioxidant effects showed by by MTT assay, lettuce germination assay, disk diffusion and DPPH method (Razavi et al., 2009);

- *quercetin-3-O-rutinoside* showed anti-proliferative activity in several leukemic cell lines (Samanta et al., 2010) and its powerful antioxidant activity was investigated (Zieliska et al., 2003 );

- *ferulic acid* have direct antitumor activity against breast cancer ( Kampa et al. 2004) and liver cancer (Lee, 2005);

- *kaempferol and its derivates* are anti-cancer agents, being used in colon cancer (Nakamura Y., 2005.);

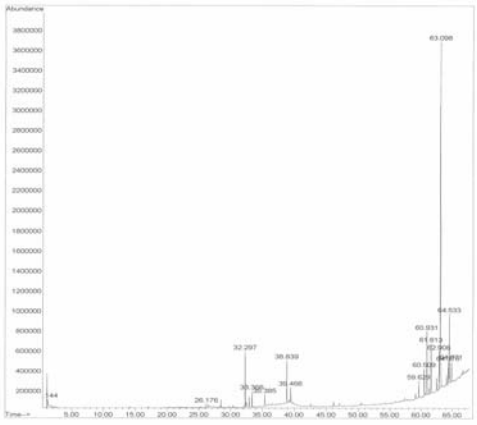

Figure 1. Chromatogram obtained for Cirsium arvense leaves

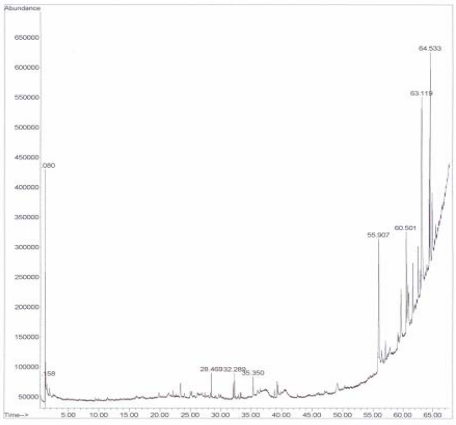

Figure 2. Chromatogram obtained for Cirsium arvense stems

Table 1

Identified compounds in the *Cirsium arvense* extract

| Compound class | Identified compound from the GC-MS library | Retention time (min) |
|---|---|---|
| Polyphenols | catechin | 7.9 |
| | cis-ferulic acid | 17.83 |
| | cafeic acid | 11.37 |
| | 3,4 - dimetoxy cinnamic acid | 11.51 |
| | quercetin-3-O-glucoside | 5.9 |
| | luteolin-7glucoside | 23.5 |
| | quercetin-3-O-rutinoside | 5 |
| | apigenin-7-(6”–metilglucuronide) | 7.3 |
| Flavonols | kaempferol-3-O-α-L-rhamnoside | 20 |
| | kaempferol-O-glucuronide | 8 |
| | kaempferol-3-O-rutinoside | 16.5 |
| | quercetin-3-O-rhamnoside | 9.48 |
| | kaempferol | 6.13 |

- *kaempferol-3-O-rutinoside* showed a remarkable decrease in systolic, diastolic, mean arterial blood pressure and heart rate (Ahmad et al., 1993);

- *3,4- Dimetoxy cinnamic acid* presents antiinflammatory and analgesic activities (Yeşilada et al., 1996);

## CONCLUSIONS

In thistle are biologically active compounds, and because of its abundance, it can be evaluated and used as a medicinal plant, finding different uses for this weed.

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# ABOUT THE XEROPHYTE MEADOWS IN OLTENIA (ROMANIA) (I)

Răduțoiu D.,[1] Costache I.,[1] Răduțoiu Amira[2]

***Keywords***: *xerophyte, area, Oltenia, Romania.*

## ABSTRACT

*The paper wants to inform those interested in Dasypyrum villosum (Haynaldia villosa) phytocoenosis, which years ago were weakly represented in this country part, that today have a considerable larger territory.*

*Factors contributing to the widening spread of these associations phytocoenosis are the zoo-anthropogenic one and the drought.*

*This aspect comes out also from the floristical composition of these phytocoenosis. Therefore is necessary to take measures for limiting the anthropogenic factor and growing of the phytodiversity in these perimeters.*

*The extending of these associations is rare met in Olteniei Plain, the piedmont hills and Subcarpathian Basin level not being in danger from this point of view (yet).*

## INTRODUCTION

Studies on the xerophyte meadows from this part of the country are met in numerous specialty papers which analyze the vegetation from these meadows (Buia et al. 1959, Buia & Păun 1960, Păun 1963, 1967, 1969, Păun et al. 1971, Dihoru et al. 1970, Roman 1962, 1974). Following the studies made by us, starting from those already existing and realising the comparation between the association tables, we observed that on certain areas, the floristic diversity decreases and the physiognomy of those meadows is nearly uniform.

This aspect has been also mentioned by the author of these paper in a research material (Răduțoiu 2006) which is analyzing a smaller area from Oltenia (Cerna of Olteț Basin).

The main purpose of this paper is to highlight, in an objective way, the structural character of the phytocoenosis of the xeric meadows in Oltenia, favored by the anthropic factor and decision taking, next to the other responsible institutions to limit this factor and preserve of as many as possible areas with a high phytodiversity.

## MATERIAL AND METHODS

The work methods used are specific for this kind of study. There have been made field trips, after a bibliographic documentation, data were collected, analyzed and compared

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with those already existing so far, after that some conclusions being noticed. The associations framing was made according to Sanda V. (2002) and the critical taxa determination from the phytocoenosis was made helped by the specialty determination books inside the country or abroad (Ciocârlan 2009, Tutin et al. 1964-1980).

## RESULTS AND DISCUTIONS

The first botanists who observe the formation of some meadows dominated by *Dasypyrum villosum (Haynaldia villosa)* are Buia, Păun, Safta and Pop (1959). The authors say that in those times, the meadows dominated by *Haynaldia villosa* occupied small surfaces, isolated islands-like (Buia et al. 1959). These meadows are met both on plane lands and sunny slopes, generally on degraded chernozem or wood reddish brown soils.

This is a southern, Mediterranean association which has been extended to us because of deforestation (Păun, 1969) during the time.

From the association tabel analyzing, presented by the group coordinated by Proffesor Buia, releases the fact that the phytocoenosis edified by this species have a high floristic composition (87 species). Among these, there are about 30 plants from Fabaceae family, which shows that, in that time, these haylands had an acceptable quality of the fodder. Subsequently, Păun M. (1969) mentioned this association around Balş, but with an obvious lower floristic composition (36 species).

In the association table presented by Sanda V. and collaborators (2007) according to Roman, the number of species is reduced to 22.

The influence of the zooanthropogenic factor in these meadows, in the last time, led to the decrease of the forage species. This fact releases form the analyze of the processed (Table 1).

*Trifolio – Dasypyretum villosae*

Table 1

| Releve number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
|---|---|---|---|---|---|---|---|---|---|
| Altitude (m) | 100 | 250 | 220 | 180 | 100 | 200 | 60 | 60 | |
| Exposure | NE-SV | S | SV | S-SV | V | S | S | S | K |
| Înclination ($^0$) | 5 | 5 | 7 | 5 | 5 | 5 | 7 | 7 | |
| Vegetation covering (%) | 90 | 100 | 90 | 98 | 90 | 90 | 90 | 90 | |
| Releve area ($m^2$) | 100 | 100 | 100 | 100 | 100 | 100 | 200 | 200 | |
| Characteristic species of the association | | | | | | | | | |
| *Dasypyrum villosum* | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | V |
| *Trifolium striatum* subsp. *tenuiflorum* | + | + | + | + | + | - | - | - | IV |
| *Trifolium incarnatum* subsp. *molinerii* | 1 | + | - | + | + | - | - | - | III |
| Thero-Airion & Thero-Airetalia | | | | | | | | | |
| *Ventenata dubia* | + | - | - | - | - | - | + | + | II |
| *Aira capillaris* | + | - | - | + | - | - | + | - | II |
| *Taeniatherum caput-medusae* | + | - | - | - | - | - | - | + | II |
| Sedo-Scleranthetea | | | | | | | | | |
| *Achillea collina* | + | + | + | + | + | - | + | + | IV |
| *Convolvulus arvensis* | + | - | + | + | + | - | - | - | III |
| *Trifolium arvense* | + | - | + | - | + | - | - | - | II |
| *Anthemis arvensis* | + | - | + | + | - | - | - | - | II |
| *Chondrilla juncea* | + | - | + | + | - | - | - | - | II |
| *Festuca valesiaca* | + | - | - | - | + | - | - | - | II |
| *Hieracium bauhinii* | + | - | + | + | - | - | - | - | II |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Echium vulgare* | - | - | + | - | + | - | - | - | II |
| Festucetalia valesiacae | | | | | | | | | |
| *Potentilla argentea* | + | + | - | + | + | - | - | - | III |
| *Sanguisorba minor* | - | + | + | - | - | - | - | - | II |
| *Fragaria viridis* | + | - | - | - | - | - | + | + | II |
| *Aegilops cylindrica* | + | 1 | + | 1 | - | - | - | - | III |
| *Centaurea apiculata* subsp. *spinulosa* | - | + | - | + | - | - | - | - | II |
| Variaesyntaxa | | | | | | | | | |
| *Vicia dasycarpa* | + | - | + | - | + | - | - | - | II |
| *Crepis setosa* | + | - | + | - | + | - | + | + | IV |
| *Rumex pulcher* | + | - | + | - | + | - | - | - | II |
| *Cardaria draba* | + | + | - | + | - | - | - | - | II |
| *Eryngium campestre* | + | - | - | + | + | - | - | - | II |
| *Falcaria vulgaris* | - | + | + | + | + | - | - | - | III |
| *Lolium perenne* | - | - | + | - | - | - | + | + | II |
| *Rhinanthus rumelicus* | - | + | - | + | - | - | - | - | II |
| *Erigeron annuus* | - | - | + | - | + | - | - | - | II |
| *Lathyrus tuberosus* | - | - | - | + | + | - | - | - | II |
| *Reseda lutea* | - | - | - | - | - | - | + | + | II |

Species present in one survey: *Carthamus lanatus* (1); *Bromus hordeaceus* (1); *Alopecurus pratensis* subsp. *pseudonigricans* (1); *Chrysopogon gryllus* (1); *Senecio vernalis* (1); *Cichorium intybus* (2); *Medicago orbicularis* (2), *Marrubrium peregrinum* (2), *Centaurea cyanus* (2), *Vicia hirsuta* (2), *Salvia nemorosa* (3), *Melilotus officinalis* (3), *Anchusa officinalis* (3), *Medicago minima* (3), *Tragopogon dubius* (3), *Verbena officinalis* (3), *Consolida regalis* (4), *Sambucus ebulus* (4), *Orlaya grandiflora* (5), *Carthamus lanatus* (5), *Crepis foetida* subsp. *rhoeadifolia* (5), *Ornithogalum pyramidale* (5), *Torilis arvensis* (7), *Symphytum officinale* (7); *Arrhenatherum elatius* (7); *Silene latifolia* subsp. *alba* (8); *Clematis integrifolia* (7);

Place and date of performing the surveys: 1. Calopăr (03.VI.2008); 2. Sopot (04.VI.2009); 3. Pietroaia (04.VI.2008); 4. Frasin (04.VI.2009); 5. Predeştii Mici (4.VI.2009); 6. Țiu-Cernăteşti (07.VI.2009); 7. Rast (03.VI.2012); 8. Ghidici (04.VI.2012).

Coenotaxonomically, we can say about the framing of these associations edified by *Dasypyrum villosum* that is made different from one author to another. According to Roman (1974) the competition of the numerous passing phytocoenosis from the eroded lands, slopes or wash torrents, that share approximately the same space led to numerous interferences and even overlapping. Nevertheless, he frames these phytocoenosis to Festucion rupicolae Soó 1964 considering that the species from this alliance are well represented.

In 1967 Boşcaiu and Resmeriță proposed a new name to this association: *Trifolio molinerii – Dasypyretum villosae* (Sanda et al. 2007). They motivate this throw the fact that the proposed species core is found in the association composition described by Buia and collaborators (1959).

Subsequently, Păun M. (1968) moved this association to Bromion erecti Br.-Bl. 1936.

In the quadrates made by Păun M. and collaborators (1971) around Sf. Elena locality is mentiond the presence of *Vicia cracca* species, with a 3-5 value of dominance. Considering this, the authors propose a facies edified by this plant.

Sanda V. and collaborators (2007) put this association in *Sedo-Scleranthetea*, *Thero-Airetalia*, *Thero-Airion* Class, considering that the phytocoenosis are installing on fallow grand, becoming trodden and represents a previous stage for the reinstallation of the detrodden associations of *Festuca valesiaca* and *Chrysopogon gryllus*, an opinion shared also by the authors of this paper.

One thing is certain, whatever the floristic composition of these phytocoenosis is, the coverage is generally of 90-100% and the physiognomy is uniform (Fig. 1, 2, 3, 4).

Fig. 1. General aspect of the meadow from the Pietroaia (orig.)

Fig. 2. General aspect of the meadow from the Frasin (orig.)

Fig. 3. General aspect of the meadow from the Tuglui (orig.)

Fig. 4. General aspect of the meadow from the Rastu Vechi (orig.)

## CONCLUSIONS

In conclusion, we can say that in the meadows edified by *Dasypyrum villosum* is present an increasing development in the last time, development which is favoured by the zooanthropogenic factor. The increasing of the area of these meadows is accompanied

frequently by the number reducing of those well forage species. Therefore is necessary to take measures for the reducing of the influence of the zooanthropogenic factor and enriching of these meadows with species with high forage value.

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# THERMOVISION - A RELIABLE METHOD APPLIED IN ENVIRONMENTAL PROTECTION IN AGRICULTURE

Roşca A.[1] , Roşca Daniela[2]

***Keywords:*** *thermovision, overheating, machines elements, environment protection*

**ABSTRACT**

*Thermovision has a wide application in various fields such as industrial maintenance, engineering (mechanical, civil, electric), aerospace, medicine, pharmacy, veterinary, agriculture and food industry, and environmental protection in agriculture. For environmental protection in agriculture Thermovision is applied as an important method for fire and explosion hazards prevention. The great benefit of Thermovision over other predictive maintenance technologies is easy infrared camera operation, and fast interprets of the results to prevent fire hazard.*

*This paper presents Thermovision examples applied for environmental protection in agriculture.*

## INTRODUCTION

With wide and general applications throughout the industrial activities, Thermovision completes, but not replace the conventional predictive maintenance methods: vibration analyses - specific applications for bearings and rotating machines' elements; acoustic level - specific applications for mechanical elements and electrical parts; tribology - specific applications for machines' mechanical elements (http://www.flir.com, 2011; Willimas, 2009).

The major advantages of Thermovision are non-contact, non-invasive, and rapid technique which could be used for online applications. With thermal cameras, it is possible to obtain temperature mapping of any particular region of interest with fast response times which is not possible with thermocouples or other temperature sensors which can only measure spot data (Vadivambal & Jayas, 2011).

The vast majority of natural and synthetic organic materials, as well as some metals, can form combustible dust. International *Industrial Fire Hazards* state that "any industrial process that reduces a combustible material and some normally noncombustible materials to a finely divided state present a potential for a serious fire or explosion." A combustible dust explosion hazard may exist in a variety of industries, including: food (dust cloud such as grain, flour, sugar, pollen, powdered milk), plastics, wood, rubber, furniture, textiles, pesticides, pharmaceuticals, dyes, coal, metals (e.g., aluminum, chromium, iron, magnesium, and zinc), and fossil fuel power generation (Eckhoff, 2003; Hatwig & Steen, 2004).

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## MATERIAL AND METHOD

International general standards for the *Prevention of Fires and Dust Explosions in Agricultural and Food Processing Facilities*, and specific standards for *Grain Handling Facilities*, and for *Explosion Prevention Systems* recommend the temperature measure with thermocouples or other temperature sensors, and for online applications in the latest versions are recommend non-contact and rapid method as Thermovision (Eckhoff, 2003; Hatwig & Steen, 2004).

In order to perform the interdisciplinary researches concerning materials strength, machines elements, fluids mechanics, food industry equipment or engineering and environment protection, in the Unconventional Technologies and Equipment for Agro-Food Industry Laboratory within Faculty of Agriculture and Horticulture in Craiova, a FLIR T200 Infrared Thermovision Camera was used (Roşca & Roşca, 2011 and 2012).

## RESULTS AND DISCUTIONS

According to international general standards for the *Prevention of Fires and Dust Explosions in Agricultural and Food Processing Facilities*, to prevent fire hazard in flour milling process, the operation temperature must not exceed 80°C.

Figure 1 presents an example describing low quality maintenance for rubber and fiberglass insertion belt transmission that operate into centralized driving of a small roller mill. It can be observed the overheating of the belt up to 81,1°C caused by poor alignment of the transmission wheels, which will cause soon the belt transmission failure (Roşca & Roşca, 2011).

During Thermovision monitoring fast increase of the sliding bearing' temperature was observed; after 40 min from the milling process starting, the maximum temperature in thermal image increased up to 151°C (figure 2).

Due to these Thermovision monitoring results, the flour milling process was proposed to be stopped. During the mechanical maintenance evaluation high ware rate of the sliding bearing was observed, therefore this machine element was replaced with a new one. After 20 min from the flour milling process start, the Thermovision monitoring revealed a proper temperature at about 48,5°C (Roşca & Roşca, 2011).

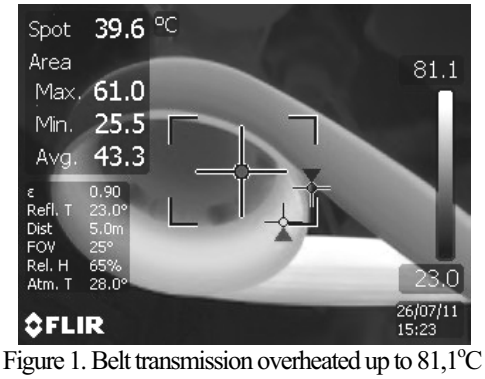


Figure 1. Belt transmission overheated up to 81,1°C

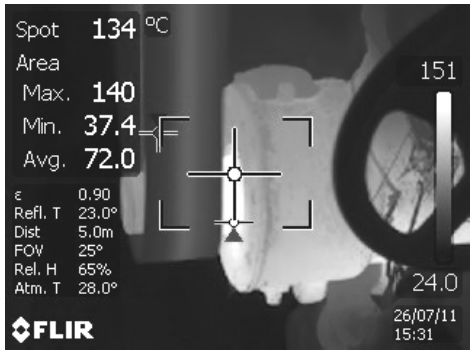


Figure2. Sliding bearing overheated at 151°C

Another example of thermovision application in engineering and environment protection concerns the influence of B20 biodiesel blend on urban buses operation and maintenance. In idle-engine mode test of the engine operated with B20 at 1150-1200RPM, maximum temperature recorded on external carter walls was up to 150,9°C, and in the engine

gas evacuation pipe area was up to 102,6$^0$C. B20/1150-1200RPM operation type causes more then 30$^0$C over-heating of engine external carter walls. In engine gas evacuation pipe area the maximum values are practically comparable/equal with the recommendations in the Technical Book of D2156HM6U type engine (Roşca & Roşca, 2012).

Figure 3 presents thermograme for B20/1150-1200RPM experimental range.

During B20Buses daily maintenance verifications, the cumulative effect of engine overheating by using B20 blend, and fast degraded parts (nitrile rubber compounds) by the prolonged exposure with B20 was observed (Roşca & Roşca, 2012).

In May and June (monthly maximum average atmospheric temperature 25,6$^0$C), the degradable set parts (nitrile rubber compounds) were necessary to be replaced 3 times. In these two months to prevent damages caused by filter plugging, the special filter provided for the tested B20Buses were 5 times cleaned (Roşca & Roşca, 2012).

In July and August, monthly maximum average atmospheric temperature raised at 32,6$^0$C, with many days with maximum daily temperature up to 44,5$^0$C. In these days the temperature on fleet asphalt (determined by using thermovision) increased up to 65,7$^0$C. To prevent fire hazard that could occurs due to fuel leakage and engine overheating, in this two month degradable set parts (nitrile rubber compounds) were replaced 8 times (once time each week). To prevent damages caused by filter plugging, in these two months, the special filter provided for the tested B20Buses were necessary to be cleaned 12 times (Roşca & Roşca, 2012).

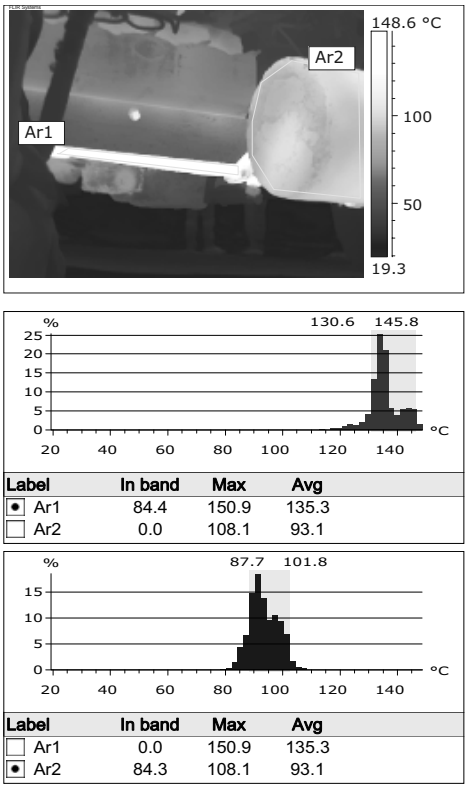


Figure 3. Thermograme for B20 / 1150-1200RPM

## CONCLUSIONS

Thermovision has a wide range of applications including, but not limited to, the ability to detect defects as driving motor loads, sliding/rotating bearing temperature (poor shaft alignment), sliding /rotating bearing wear (lubrication failure or pitted damaged), imbalanced load of the machine elements during operation that can cause fire / explosion hazard in industrial process that could cause important implications in environment protection.

Potential use of thermal imaging in environment protection could include Thermovision in monitoring programmes according with international general standards for the *Prevention of Fires and Dust Explosions in Agricultural and Food Processing Facilities*.

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# HARMFUL AND BENEFICIAL SPECIES AND THE RELATIONSHIPS BETWEEN THEM WITHIN THE ECOSYSTEM NICOLAE ROMANESCU PARK

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***Key words:*** *harmful species, predators species, parasites species*

## ABSTRACT

*From our observation regarding the harmful and beneficial species and the trophic relationships between them, made in Nicolae Romanescu Park, we have identified a number of 108 pest species. Most of the insect species identified belonged to the Coleoptera order (30 species), followed by Lepidoptera (24 species), Homoptera (13 species) Heteroptera (11 species). Vertebrates harmful species from Aves class were represented by 7 species and those from the Mamalia class by 5 species. There have been also identified a number of 39 beneficial species. Most of the beneficial insect species identified belonged to the order Coleoptera (7 species) followed by the Diptera (6 species) and Hymenoptera (4 species). Vertebrates beneficial species from Aves class were represented by 8 species and those of the Mamalia class by 3 species. Amphibia class was represented by 3 species and Reptilia class by 3 species.*

## INTRODUCTION

It is obvious that nature can take care of herself. In a balanced ecosystem, as tend to be all gardens and parks that follow the laws of nature, pests are not usually a problem because there is an adjustment of populations, natural control, where internal factors of these populations intervene (endogenous factors) as well external factors (exogenous).

The endogenous factors are represented by the population polymorphism, which imply the differentiation of individuals from these populations, on special categories phenotypically and genotypically differentiated by the nterrelationships between individuals of different morpheme and the functions performed by these morpheme.

Populations exogenous factors are as for other organisms, represented by abiotic factors: physical, chemical, mechanical and biotic ones: predators, parasites, pathogens, also called "natural enemies", antagonists, as well food and competition (Toncea I. , 2012)

Even if it is a man-made ecosystem, in Nicolae Romanescu Park chemical treatments are usually only against mosquitoes and ticks or hairy caterpillar when appropriate, so beneficial species (predators and parasites) ensure efficient pest control close to the naturally model (without human intervention).Classical biological control (natural enemy introductions) has long served as a paradigm for the role of predators and

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parasitoids in insect herbivore population dynamics, and it is widely held that there is no fundamental difference between successful biological control and the action of native natural enemies 'natural control' (Bradford A. Hawkins et all, 1999). In this paper we have propose to inventory the harmful and beneficial species present in Craiova Nicolae Romanescu Park ecosystem and the trophic relationships between them.

## MATERIALS AND METHODS

Experiments were conducted during May 2011 - July 2012 in Nicolae Romanescu Park in Craiova. To determine the structure of the harmful and beneficial populations were made collection of material using various means and methods: directly by hand from plants or soil, frame metric, soil surveys and soil surface collected with entomological net, visual inspection, collection with sticky traps for flying insects, analyzing samples with binocular magnifier glass directly in the field or laboratory.After collecting, the biological material was analyzed and determined with the binocular magnifier glass using the Identification Manual (Panin L, 1951, Chatened du Gaetan, 1990, Chinery M., 1998, Godeanu S.P., 2002, Szabó D. Zoltán et all 2010, Bertel Bruun et all 2011). For as little impact on the ecosystem we have preferred to capture images with the camera than to capture live specimens were subsequently removed from their natural environment.

## RESULTS AND DISCUSSION

There were identified a number: 108 pest species (Table 1), systematically placed in 12 orders (belonging to 4 classes: *ARACHNIDA, INSECTA, AVES, MAMALIA*.

Table nr.1

Entomofauna and harmful vertebrate identified during research

| Nr. crt | Order | Species |
|---|---|---|
| 1 | ***ACARI*** | *Panonychus ulmi* Koch. |
| 2 | | *Tetranychus urticae* Koch. |
| 1 | ***ORTHOPTERA*** | *Gryllus campestris* L. |
| 2 | | *Gryllus desertus* L |
| 3 | | *Ephippiger ephippiger* Fieb. |
| 4 | | *Caliptamus italicus* L. |
| 5 | | *Dociostaurus maroccanus* Thunb. |
| 6 | | *Tetigonia viridisima* |
| 7 | | *Locusta migratoria L.* |
| 8 | | *Gryllotalpa gryllotalpa* |
| 1 | ***DERMAPTERA*** | *Forficula auricularia* L. |
| 1 | ***THYSANOPTERA*** | *Haplothrips tritici* |
| 2 | | *Limothrips cerealium Hal.* |
| 1 | ***HETEROPTERA*** | *Lygus pabulinus* L. |
| 2 | | *Lygus pratensis* L. |
| 3 | | *Dolycoris bacarum* L. |
| 4 | | *Pentatoma rufipes* L. |
| 5 | | *Eurygaster integriceps L.* |
| 6 | | *Eurygaster maura L.* |
| 7 | | *Eurygaster austriaca Schr.* |
| 8 | | *Aelia acuminata L.* |
| 9 | | *Aelia rostrata Boh.* |

| Nr. crt | Order | Species |
|---|---|---|
| 10 | | *Graphosoma lineata* |
| 11 | | *Pyrocoris apterus* |
| 1 | | *Quadraspidiotus perniciosus* Comst. |
| 2 | | *Cicada plebeja* |
| 3 | | *Ceresa bubalus* L. |
| 4 | | *Eulecanium corni* Bch. |
| 5 | | Psylla mali Schmdt. |
| 6 | | *Aphis pomi De Geer.* |
| 7 | ***HOMOPTERA*** | *Aphis fabae L.* |
| 8 | | Eriosoma lanigerum Hausm. |
| 9 | | *Aulacaspis rosae* |
| 10 | | *Macrosiphon rosae* |
| 11 | | *Myzodes persicae* |
| 12 | | *Hyalopterus pruni* |
| 13 | | *Myzus cersi* |
| 1 | | *Vespa vulgaris* L. |
| 2 | ***HYMENOPTERA*** | *Vespa germanica* L. |
| 3 | | *Hoplocampa spp.* |
| 1 | ***COLEOPTERA*** | *Melolontha melolontha* L |
| 2 | | *Melolontha hippocastani* F. |
| 3 | | *Polyphylla fullo* F. |
| 4 | | *Anomala solida* Er. |
| 5 | | *Anomala vitis* F. |
| 6 | | *Anomala dubia* Scop. |
| 7 | | *Amphimalon solstitialis* L. |
| 8 | | *Rhyzothrogus aequinoctialis* Herb. |
| 9 | | *Anoxia orientalis* L. |
| 10 | | *Lethrus apterus* Laxum. |
| 11 | | *Phyllopertha horticola* L. |
| 12 | | *Haltica ampelophaga* Gue.-Men. |
| 13 | | *Crioceris asparagi* |
| 14 | | *Chrysomella violacea* |
| 15 | | *Byctiscus betulae* L. |
| 16 | | *Otiorrhynchus ligustici* L. |
| 17 | | *Otiorrhynchus sulcatus* F. |
| 18 | | *Cetonia aurata* Oliv. |
| 19 | | *Chrysomella mentharsti* |
| 20 | | *Ruguloscolytus rugulosus* Ratz. |
| 21 | | *Zabrus tenebrioides Goeze.* |
| 22 | | *Anisoplia segetum Herb.* |
| 23 | | *Anisoplia austriaca Herb.* |
| 24 | | *Anisoplia agricola Poda.* |
| 25 | | *Agriotes obscurus L.* |
| 26 | | *Opatrum sabulosum L.* |
| 27 | | *Lilioceris lilii L.* |

| Nr. crt | Order | Species |
|---|---|---|
| 28 | | *Anthonomus pomorum L.* |
| 29 | | *Epicometis hirta Poda.* |
| 30 | | *Oxythyrea funesta Poda.* |
| 31 | | *Rhynchites bacchus L.* |
| 1 | ***LEPIDOPTERA*** | *Aporia crataegi L.* |
| 2 | | *Sphynx convolvuli* |
| 3 | | *Pieris brassicae* |
| 4 | | *Pieri napi* |
| 5 | | *Pieris rapae* |
| 6 | | *Hyphantria cunea Drurry.* |
| 7 | | *Hyponomeuta malinella Zell.* |
| 8 | | *Arginis pahia L.* |
| 9 | | *Cydia pomonella L.* |
| 10 | | *Laspeyresia funebrana* |
| 11 | | *Euproctis chrysorrhoea L.* |
| 12 | | *Malacosoma neustria L.* |
| 13 | | *Operophtera brumata L.* |
| 14 | | *Hibernia defoliaria Cl.* |
| 15 | | *Noctua pronuba* L. |
| 16 | | *Amphipyra tragopoginis* L. |
| 17 | | *Acronicta rumicis* Hb. |
| 18 | | *Euxoa nigricans* L. |
| 19 | | *Phlogophora meticulosa* L. |
| 20 | | *Helicoverpa armigera* |
| 21 | | *Zeuzera pyrina* L. |
| 22 | | *Vanessa polychloros* L. |
| 23 | | *Vanessa urticae* |
| 24 | | *Vanessa io* |
| 1 | ***DIPTERA*** | *Musca domestica* |
| 1 | ***AVES/ PASSERIFORMIS*** | *Passer domesticus domesticus* L. |
| 2 | | *Corvus frugilegus frugilegus L.* |
| 3 | | *Corvus corone cornix L.* |
| 4 | | *Pica pica pica L.* |
| 5 | | *Sturnus vulgaris balcanicus* But et Harms |
| 6 | | *Garrulus glandarius glandarius* |
| 7 | | *Nucifraga caryocatactes* |
| 1 | ***MAMALIA /RODENTIA*** | *Microtus arvalis laevis* Pall. |
| 2 | | *Lepus europaeus* Pall |
| 3 | | *Citellus citellus L.* |
| 4 | | *Cricetus cricetus L.* |
| 5 | | *Arvicola terrestris L.* |

As shown in the table most insect species identified belong to the Order Coleoptera (30 species), followed by Lepidoptera (24 species), Homoptera (13 species) and Heteroptera (11 species).

Harmful vertebrate from Aves class were represented by 7 species and those of the Mamalia class by 5 species.There has been also identified a number of 39 useful species (table no. 2), belonging to 6 classes: ARACHNIDA, INSECTA, AVES, MAMALIA, AMPHIBIA şi REPTILIA.

Table nr.2

Entomofauna and beneficial vertebrate identified during research

| Nr. crt | Class/Order | Species |
|---|---|---|
| 1 | ***ARACHNIDA/ACARI*** | *Typhlodromus* spp. |
| 2 | | *Amblyseius* spp. |
| 1 | ***INSECTA/ORTHOPTERA*** | *Mantis religiosa* |
| 1 | ***INSECTA/ NEUROPTERA*** | *Crisopa carnea Ste.* |
| 2 | | *Crisopa perla Steph.* |
| 1 | ***INSECTA/ HYMENOPTERA*** | *Scolia flavifrons* |
| 2 | | *Vespa crabro* |
| 3 | | *Bombyx terestris* |
| 4 | | *Syrcopa violacea* |
| 1 | ***INSECTA/ COLEOPTERA*** | *Adalia decimpunctata L.* |
| 2 | | *Adalia bipunctata L.* |
| 3 | | *Coccinella 7 punctata L* |
| 4 | | *Carabus cancelatus L.* |
| 5 | | *Carabus ulrichi L.* |
| 6 | | *Carabus violaceus L.* |
| 7 | | *Calosoma sycophanta L.* |
| 1 | ***INSECTA/ DIPTERA*** | *Syrphus ribesii L.* |
| 2 | | *Syrphus torvus L.* |
| 3 | | *Episyrphus balteatus* De Geer |
| 4 | | *Metasyrphus corollae* F. |
| 5 | | *Scaeva albmaculata* Macq. |
| 6 | | *Scaeva pyrastri* L. |
| 1 | ***AVES*** | *Parus parus* |
| 2 | | *Parus caeruleus* |
| 3 | | *Parus major* |
| 4 | | *Picus viridis* |
| 5 | | *Picus picus* |
| 6 | | *Phasanius colchicus* |
| 7 | | *Perdix perdix* |
| 8 | | *Athene noctua* |
| 1 | ***MAMALIA*** | *Pipistrellus pipistrellus* |
| 2 | | *Talpa europaea* |
| 3 | | *Erinaceus europaeus* |
| 1 | ***AMPHIBIA*** | *Bufo bufo* |
| 2 | | *Rana esculenta* |
| 3 | | *Emis orbicularis* |
| 1 | ***REPTILIA*** | *Lacerta agilis* |
| 2 | | *Lacerta viridis* |
| 3 | | *Natrix natrix* |

As it can be observed most beneficial insect species identified belonged to the Order Coleoptera (7 species) followed by the Diptera (6 species) and Hymenoptera (4 species). Beneficial vertebrates from Aves class were represented by 8 species, and those of Mamalia class by 3 species. Amphibia class was represented by 3 species and Reptilia class by 3 species.

It can be seen that although the number of species of pests (108) identified during the research is higher than of the beneficial species (39), the latter manage to control the harmful populations. This is due to the fact that some harmful species under certain conditions (larval growth, raising fledgeling, etc.). can consume different harmful organisms . Such is earwig - Forficula auricularia, which feeds on small insects and, of corvids and house sparrow that during the fledgeling feeding consume harmful insects feeding.

In anthropogenic agroecosystems human intervention through chemical methods to limit the pest attacks, resulting in serious disruption of beneficial organisms activity having a negative influence, due to direct exposure to pesticide action and indirect action due to the active substance ingested with the prey.

The ecosystem Nicolae Romanescu Park from Craiova is an anthropogenic ecosystem but chemical interventions against pests, which directly disturb the beneficial organisms activity (predators and parasites) are very low.

They are limited to chemical treatment to control mosquitoes, ticks and some seasonal defoliators (hairy caterpillar - Hyphantria cunea) or application of treatments only in areas where there has been recorded damage caused by pests.

Thus trophic activity of the predators takes place in conditions almost natural and that's why the population of predators provide a reasonable level of pest control.

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# AN EXAMPLE OF OSSERMAN MANIFOLDS WITH NILPOTENT JACOBI OPERATOR

Șterbeți C.[1]

***Keywords****: Jacobi operator, semi-Riemannian metric*

**ABSTRACT**

*In this paper we study the Jacobi operator in semi-Riemannian geometry. We define a particular metric in the neutral (2,2) semi-Riemannian manifold which generate an Osserman manifold with Jacobi operator 2-nilpotent.*

Let $(M, g)$ be a pseudo-Riemannian manifold of signature $(p,q)$ and dimension $m = p + q$ and $R(\cdot,\cdot)$ be the associated Riemann curvature operator.

The Jacobi operator $R_X : Y \to R(Y, X)X$ is a self-adjoint operator.

Let $S^{\pm}(M, g) = \{X \in TM / g(X, X) = \pm 1\}$ be the bundles of unit spacelike and unit timelike vectors, respectively. One says that $(M, g)$ is spacelike Osserman or timelike Osserman if the eigenvalues of Jacobi operator are constant on $S^{+}(M, g)$ or on $S^{-}(M, g)$ respectively. These concepts are equivalent if $p \geq 1$ and $q \geq 1$, so such manifolds are said to be Osserman.

We define the family of metric tensors of signature (2,2) on $\mathbb{R}4$ by

$$g_V = y_1 \frac{\partial V}{\partial x_1}(x_1, x_2)dx_1 \otimes dx_1 - y_2 \frac{\partial V}{\partial x_2}(x_1, x_2)dx_2 \otimes dx_2 + \\ + a[dx_1 \otimes dx_2 + dx_2 \otimes dx_1] + b[dx_1 \otimes dy_1 + dy_1 \otimes dx_1 + dx_2 \otimes dy_2 + dy_2 \otimes dx_2] \tag{1.1}$$

where a and b are real constants and $V \in \mathbb{C}^1(\mathbb{R}^2)$.

We shall use the notations

$$\frac{\partial}{\partial x_i} = \partial_i^x\,,\ \frac{\partial}{\partial y_i} = \partial_i^y\,,\ \frac{\partial V}{\partial x_i} = V_{;i}\,,\ \frac{\partial^2 V}{\partial x_i \partial x_j} = V_{;ij}\,,\ \frac{\partial^3 V}{\partial x_i \partial x_j \partial x_k} = V_{;ijk}\,,\ i, j, k = 1,2.$$

The nonvanishing components of covariant derivates are:

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$$\begin{cases} \nabla_{\partial_1^x}\partial_1^x = -\frac{1}{2b}V_{;1}\partial_1^x + \left(\frac{1}{2b}y_1V_{;11} + \frac{1}{2b^2}y_1V_{;1}^2\right)\partial_1^y + \left(-\frac{1}{2b}y_1V_{;12} + \frac{a}{2b^2}V_{;1}\right)\cdot\partial_2^y \\ \nabla_{\partial_1^x}\partial_2^x = \frac{1}{2b}y_1V_{;12}\partial_1^y - \frac{1}{2b}y_2V_{;12}\partial_2^y \\ \nabla_{\partial_1^y}\partial_1^y = \frac{1}{2b}V_{;1}\partial_1^y \\ \nabla_{\partial_2^x}\partial_2^x = \frac{1}{2b}V_{;2}\partial_2^x + \left(\frac{1}{2b}y_2V_{;12} - \frac{a}{2b^2}V_{;2}\right)\partial_1^y + \left(-\frac{1}{2b}y_2V_{;22} + \frac{1}{2b^2}y_2V_{;2}^2\right)\cdot\partial_2^y \\ \nabla_{\partial_2^x}\partial_2^x = -\frac{1}{2b^2}V_{;2}\partial_2^y \end{cases} \quad (1.2)$$

Using these formulas we obtain that the nonvanishing components of curvature tensor for $(\mathbb{R}^4, g_V)$ are

$$\begin{cases} R(\partial_1^x,\partial_2^x)\partial_1^y = -\frac{1}{2b}V_{;12}\partial_1^y \\ R(\partial_1^x,\partial_2^x)\partial_2^y = -\frac{1}{2b}V_{;12}\partial_2^y \\ R(\partial_1^x,\partial_1^y)\partial_1^x = \frac{1}{2b}V_{;12}\partial_2^y \\ R(\partial_1^x,\partial_1^y)\partial_2^x = -\frac{1}{2b}V_{;12}\partial_1^y \\ R(\partial_2^x,\partial_2^y)\partial_1^x = \frac{1}{2b}V_{;12}\partial_2^y \\ R(\partial_2^x,\partial_2^y)\partial_2^x = -\frac{1}{2b}V_{;12}\partial_1^y \\ R(\partial_1^x,\partial_2^x)\partial_1^x = \frac{1}{2b}V_{;12}\partial_1^x - \frac{1}{2b^2}y_1V_{;1}V_{;12}\partial_1^y + \frac{1}{4b^3}[2b^2y_1V_{;122} - 2b^2y_2V_{;112} + \\ \qquad + b(-y_1V_{;2} - y_2V_{;1} - 2a)V_{;12} + aV_{;1}V_{;2}]\partial_2^y \\ R(\partial_1^x,\partial_2^x)\partial_2^x = \frac{1}{2b}V_{;12}\partial_2^x + \frac{1}{2b^2}y_2V_{;2}V_{;12}\partial_2^y - \frac{1}{4b^3}[2b^2y_1V_{;122} - 2b^2y_2V_{;112} + \\ \qquad + b(-y_1V_{;2} - y_2V_{;1} + 2a)V_{;12} + aV_{;1}V_{;2}]\partial_1^y \end{cases} \quad (1.3)$$

Now, if $X = \alpha_1\partial_1^x + \alpha_2\partial_2^x + \alpha_3\partial_1^y + \alpha_4\partial_2^y$ is a vector field on $\mathbb{R}^4$, then the Jacobi operator $R_X$ has associated matrix on basis $\{\partial_1^x, \partial_2^x, \partial_1^y, \partial_2^y\}$ given by

$$R_X = \begin{pmatrix} A & 0 \\ B & {}^tA \end{pmatrix} \quad (1.4)$$

where $A = \frac{1}{2b}V_{;12}\begin{pmatrix} \alpha_1\alpha_2 & -\alpha_1^2 \\ \alpha_2^2 & -\alpha_1\alpha_2 \end{pmatrix}$ (1.5)

From the form of Jacobi operator, we can obtain that for every non-zero field on $\mathbb{R}4$, the associated characteristic polynomial satisfy $p_\lambda(R_X) = \det(R_X - \lambda I_4) = \lambda^4$, thus all eigenvalues are vanish and ($\mathbb{R}4$, gV) is Osserman.

More than that,

$$R_X^2 = \begin{pmatrix} 0 & 0 \\ BA + {}^tAB & 0 \end{pmatrix} = \frac{1}{4b^3} g(X,X) V_{;12}^2 \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ -\alpha_1^2 & \alpha_1\alpha_2 & 0 & 0 \\ \alpha_1\alpha_2 & -\alpha_2^2 & 0 & 0 \end{pmatrix} \qquad (1.6)$$

$$R_X^3 = \begin{pmatrix} 0 & 0 \\ \left(BA + {}^tAB\right) A & 0 \end{pmatrix} = 0 \qquad (1.7)$$

for every vector field X on $\mathbb{R}^4$.

From (1.3) we can see that the function $\Phi(x_1, x_2, y_1, y_2) = 2b^2(y_1 V_{;122} - y_2 V_{;112}) + aV_{;1}V_{;2}$ determine the minimal polynomial in the points where $V_{;12} = 0$. More exactly,

a) $(\mathbb{R}^4, g_V)$ has null constant curvature $(m_\lambda(R_X) = \lambda)$ when $\Phi = 0$;

b) the minimal polynomial $m_\lambda(R_X) = \lambda^2$ in the points where $\Phi \neq 0$.

For the situation when $V_{;12} \neq 0$, the relation (1.7) show that the minimal polynomial is $m_\lambda(R_X) = \lambda^3$.

Example. The 4-dimensional space (*M*, *g*), where the metric tensor

$$g_{(x_1, x_2, y_1, y_2)} = \frac{y_1}{x_1} dx_1 \otimes dx_1 + \frac{y_2}{x_2} dx_2 \otimes dx_2 + \alpha dx_1 \otimes dx_2 + dx_1 \otimes dy_1 + dx_2 \otimes dy_2 \quad (1.8.)$$

is Osserman, with associated Jacobi operator 2-nilpotent.

(Here we assume that *M* is an open set in $\mathbb{R}^4$ with $x_1 \neq 0$, $x_2 \neq 0$)

More than that, (*M*, *g*) is locally symmetric space.

Remarks:

1. In Sterbeti C. (2005), we define a semi-Riemannian metric of neutral signature (2,2).

$$g_{(f_1, f_2, h)} = y_1 f_1(x_1) dx_1 \otimes dx_1 + y_2 f_2(x_2) dx_2 \otimes dx_2 + h(x_1, x_2)[dx_1 \otimes dx_2 + dx_2 \otimes dx_1] + \\ + a[dx_1 \otimes dy_1 + dy_1 \otimes dx_1 + dx_2 \otimes dy_2 + dy_2 \otimes dx_2] \qquad (1.9)$$

where $a \in \mathbb{R}^*$ and $f_1, f_2, h$ are smooth real values functions.

The coefficients of $g_{(f_1,f_2,h)}$ depend on x and y.

In the same paper, we give the following result.

The manifold ($\mathbb{R}4$, $g_{(f_1,f_2,h)}$) is a locally symmetric space if and only if the functions f1, f2, h are solutions of the following partial differential equations in $\mathbb{R}2$:

$$\frac{\partial \varphi}{\partial x_k} + \frac{f_k}{2a}\varphi = 0, \quad k = 1,2 \tag{1.10}$$

where we note

$$\varphi(x_1, x_2) = \frac{1}{a}\left[\frac{\partial^2 h}{\partial x_1 \partial x_2} + \frac{1}{2a} f_2 \frac{\partial h}{\partial x_1} + \frac{1}{2a} f_1 \frac{\partial h}{\partial x_2} + \frac{1}{4a^2} f_1 f_2 h\right] \tag{1.11}$$

2. The metric (1.1) can be obtain from the metric (1.9), if $V$ has the form $V(x_1, x_2) = F_1(x_1) + F_2(x_2)$ and $h(x_1, x_2) = const$ in (1.9).

Then $\varphi(x_1, x_2) = \frac{h}{4a^3} f_1(x_1) f_2(x_2)$.

The conditions (1.10) for locally symmetry are in this case

$$\begin{cases} f_2\left(f_1' + \frac{1}{2a} f_1^2 h\right) = 0 \\ f_1\left(f_2' + \frac{1}{2a} f_2^2 h\right) = 0 \end{cases} \tag{1.12}$$

From (1.12) we can see easily that (M, g), with g defined in (1.8) is locally symmetric space for a convenient choice of $\alpha$.

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# MEDICINAL PLANTS WITH DIURETIC PROPERTIES FROM OLTENIA FLORA

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***Key words****: medicinal plants, diuretic action, ethnopharmacology, Oltenia, modern therapy*

**ABSTRACT**

*In this paper, the authors present, in taxonomic order, 22 species with diuretic properties from Oltenia flora: Equisetum arvense, Juniperus communis, Betula pendula, Saponaria officinalis, Cerasus avium, Cerasus vulgaris, Ononis spinosa, Fraxinus excelsior, Galium verum, Sambucus nigra, Lamium album, Origanum vulgare, Hieracium pilosella, Taraxacum officinale, Eupatorium cannabinum, Inula helenium, Centaurea cyanus, Arctium lappa, Cichorium intybus, Xanthium spinosum, Elymus repens, Zea mays.*

## INTRODUCTION

In the ethnopharmacology of the South-West of Romania, the medicinal plants are used from centuries ago. Ovidius and Dioscoride mention some medicinal plants used by Dacian people (Tiță *et al.*, 2009).

In Oltenia region, about 50% of the population is engaged with farming. For this reason, the memory of traditionally medicine still survives, in spite of the progress of pharmacological sciences, remaining a common heritage to be used when necessary.

A remarkable diversity could be observed in the Romanian flora: 4000 species, 900 of them used in traditional medicine (Ciocârlan, 2000).

In the region of Oltenia, we found over 2300 species and 600 of them are used for their medicinal properties (Tiță and Năstase, 1997).

In recent years, phytotherapy has been reconsidered and an extensive research began especially on the identification and study of diuretic products.

Diuretic herbs contain different active principles adjuvant in the treatment of edema: flavonosides, saponosides, pentacyclic terpenes, allantoin, essential oils, mineral salts (potassium).

The medicinal products with diuretic properties are recommended mainly for cardiovascular (heart failure, hypertension), urinary and metabolic (obesity, gout) diseases

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(Bojor and Popescu, 2009).

The aim of our study is to review the use of some medicinal plants for diuretic properties in the ethnopharmacology of Oltenia region.

## MATERIALS AND METHODS

The ethnopharmacological inventory of some medicinal plants with diuretic effect form Oltenia flora refers to the botanical description, ecological and chorological data, chemical composition, action and therapeutic uses of the vegetal products. The plant specimens were collected and identified following "The Illustrated Flora of Romania. *Pteridophyta et Spermatophyta*" (Ciocârlan, 2000). Voucher specimens were deposited in the herbarium of Pharmacognosy & Phytotherapy Department, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova.

## RESULTS AND DISCUSSION

The paper reports, in taxonomic order, on 22 medicinal plants with diuretic properties of 12 families that are mostly used in ethnopharmacology of Oltenia region, thus confirming the usefulness of medicinal plants for modern therapy.

*Equisetum arvense* L., horsetail (*Equisetaceae*) grows on wet and marshy soils as weed in crop fields in the plains to the mountains (Ciocârlan, 2000; Tiță, 2008). *Equiseti herba*, sterile assimilatory stems contain saponosides, flavonosides, essential oil and silicon salts. The vegetal product has diuretic action for the treatment of renal disorders, chronic polyarthritis, rheumatism, chronic skin ulcers and for the enhancement of peripheral circulation (Istudor, 1998; Valnet, 2003; Oniga, 2007; Wright *et al.*, 2007; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Juniperus communis* L., juniper (*Cupressaceae*) is a shrub with a height of up to 3–5 m. It is spread throughout the Carpathians at 700–1500 m altitude. Three-grouped acicular leaves are vertically arranged (Ciocârlan, 2000; Tiță, 2008). The medicinal product *Juniperi fructus* (*baccae*) contain essential oil, tannin, bitter principles (juniperin), sugars, pectins, resins, organic acids. It has diuretic, antiseptic and diaphoretic properties (Ciulei *et al.*, 1993; Istudor, 2001; Oniga, 2007; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Betula pendula* Roth sin. *B. verrucosa* Ehrh., Birch (*Betulaceae*) is a tree that can reach 20 m high, in hilly or mountain areas up to 1600 m altitude (Ciocârlan, 2000; Tiță, 2008). Diuretic, antimicrobial and antirheumatic properties of *Betulae folium* are due to the content of polyphenols, flavonoids, tannins and terpenes; thus, edema and albuminuria are reduced and diuresis is increased (Ciulei *et al.*, 1993; Istudor, 1998; Valnet, 2003; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Saponaria officinalis* L., common soapwort (*Caryophyllaceae*) is a perennial plant of 50–70 cm tall. It grows along riversides, roads and is cultivated for ornamental purposes (Ciocârlan, 2000; Tiță, 2008). Triterpene saponosides from *Saponariae rubrae radix* decoction are used for their expectorant, diuretic and depurative actions (Ciulei *et al.*, 1993; Istudor, 1998; Valnet, 2003; Oniga, 2007; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Cerasus avium* (L.) Moench sin. *Prunus avium* L., sweet cherry and ∗ *Cerasus vulgaris* Miller sin. *Prunus cerasus* L., sour cherry (*Rosaceae*) are cultivated trees for their fruits rich in sugars and vitamins important in food industry (Ciocârlan, 2000; Tiță, 2008). *Cerasorum stipes* (flower stalks) contain tannin, flavonosides, proanthocyanidins, saponins, potassium salts. It has diuretic and antilithiasic properties in different formulas of diuretic

teas (Ciulei *et al.*, 1993; Valnet, 2003; Bojor and Popescu, 2009; Bruneton, 2009).

*Ononis spinosa* L., rabbit bone, spiny restharrow (Fabaceae) is an undergrowth, enduring, thorny plant of 30–70 cm high. The rhizome continues with a 25–30 cm long root. It grows in grasslands, steppes, sandy places, briers, along riversides (Ciocârlan, 2000; Țiță, 2008). Triterpenoid tetracylic saponins (*α*- and *β*-onocerin or onokol), isoflavonosides (ononin or formonetin-7-O-glucoside), sterols (*β*-sitosterol), essential oil (*trans*-anethole, carvone, menthole), glycosidated phenyl-benzyl-ketones (onospin, with aglycone ononetin), tannin, organic acids, sugars, and mineral salts have been previously isolated from *Ononidis radix*. The medicinal product has diuretic-natriuretic, anti-inflammatory, antibacterial and cholagogue action, in the composition of diuretic teas for the treatment of renal lithiasis, dropsy, inflammation of the bladder and kidney, rheumatism and skin disorders (Ciulei *et al.*, 1993; Istudor, 1998; Valnet, 2003; Oniga, 2007; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Fraxinus excelsior* L., ash (*Oleaceae*) is a tree that reaches up to 20 m height. It is common in lowland and hill forests but is also grown in parks, on roadsides. A dark rhytidome covers the tree trunk. It has black buds and opposite leaves, with 7–15 oddly pinnate, sessile, hairless, oval lanceolate, acuminate and toothed leaflets, with slightly asymmetrical base. Arranged in axillary hanging panicles, polygamous, hermaphrodite or unisexual flowers appear before the leaves. The hermaphrodite flower consists of 2–3 stamens and a bicarpellate gynoecium (Ciocârlan, 2000; Țiță, 2008). The medicinal product *Fraxini folium* contain coumarin derivatives (fraxoside, fraxetol, isofraxetol), flavonosides (rutoside), catechic tannin, pentacyclic triterpene acids (ursolic acid), mannitol, inositol, glucose, organic acids, essential oil, gums. It has diuretic, diaphoretic, laxative, analgesic, anti-inflammatory and uricosuric actions for the treatment of gout, arthritis, arthrosis, rheumatism, lithiasis (Ciulei *et al.*, 1993; Istudor, 1998; Valnet, 2003; Wright *et al.*, 2007; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Galium verum* L., lady's bedstraw, yellow bedstraw (*Rubiaceae*) is a perennial herb of 60–80 cm tall, frequently in steppe areas and meadows. The erect, cylindrical stem bears golden-yellow flowers, with a pleasant honey fragrance (Ciocârlan, 2000; Țiță, 2008). Flavones, tannins and coumarins, identified in *Galii herba*, the flowering aerial part of the species, have diuretic, antirheumatic and antiseptic properties (Ciulei *et al.*, 1993; Istudor, 2001; Valnet, 2003; Oniga, 2007; Bruneton, 2009).

*Sambucus nigra* L., elder (*Caprifoliaceae*) is a deciduous shrub or a small tree up to 2–7 m height, frequently on forests' edges, bushes, water meadows (Ciocârlan, 2000; Țiță, 2008). *Sambuci flos* contains cyanide glycosides (sambunigrin, prunasin), flavones (rutoside), saponosides, essential oil, heteroglycans. It has antitussive, diuretic, expectorant, diaphoretic, emollient, immunostimulatory and antirheumatic effect (Ciulei *et al.*, 1993; Istudor, 1998; Valnet, 2003; Wright *et al.*, 2007; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Lamium album* L., white nettle (*Lamiaceae*) is a perennial species that grows in shady places or forests' edges (Ciocârlan, 2000; Țiță, 2008). Iridoids, saponosides, tannin, essential oil, flavonosides, mucilages, carotenoids, vitamins (C, K) and potassium salts are the main active principles of *Lamii herba* and *Lamii flos*, the flowering aerial part or only the flowers. Because of their anti-inflammatory, sedative, expectorant, diuretic, depurative, cicatrizing and vasoconstrictive actions, the medicinal products are recommended for bronchitis, gastritis, vascular, urinary, skin and female genital disorders (Ciulei *et al.*, 1993; Istudor, 2001; Valnet, 2003; Oniga, 2007; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Origanum vulgare* L., oregano (*Lamiaceae*) is a perennial herb of 30–50 cm tall, growing in steppes, shrubs, forest edges, crops. The stem is erect, branched in the upper

part, reddish, pubescent. The leaves are ovate, opposite. The flowers have pink-purple corolla, arranged in corymb inflorescences (Ciocârlan, 2000; Tiță, 2008). The medicinal product *Origani herba*, harvested from the aerial part of the species, contains essential oil, tannin, flavones, anthocyanins, pentacyclic triterpene acids, sugars, vitamins, organic acids, mineral salts. It has antiseptic, stomachic, diuretic, and spasmolytic action for the treatment of respiratory and digestive infections, spastic enterocolitis and nervousness (Ciulei *et al.*, 1993; Istudor, 2001; Oniga, 2007; Bruneton, 2009; Mencinicopschi *et al.*, 2009; Tiță *et al.*, 2011a).

*Hieracium pilosella* L., mouse-ear hawkweed (*Asteraceae*) is a perennial herb of 10–30 cm tall, common in oak forests and sunny meadows (Ciocârlan, 2000; Tiță, 2008). Flavones, coumarins and phenylpropane derivatives from *Pilosellae herba* show diuretic saluretic, uricosuric, anti-inflammatory and antibacterial properties recommended in renal insufficiency, oliguria, albuminuria (Valnet, 2003; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Taraxacum officinale* Weber ex Wiggers, dandelion (*Asteraceae*) is a common perennial herb. Lanceolate leaves are arranged in a runcinate rosette (Ciocârlan, 2000; Tiță, 2008). *Taraxaci radix* contains bitter sesquiterpenes, flavonoids, sterols, carotenoids, inulin, vitamins, mineral salts. It has bitter tonic, cholagogue, diuretic, diaphoretic, laxative and peristaltic properties, used in anorexia, cholecystitis, hypoacid gastritis, renal calculi (Ciulei *et al.*, 1993; Istudor, 2001; Oniga, 2007; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Eupatorium cannabinum* L., hemp agrimony (*Asteraceae*) is a perennial herb growing in the plains, hills and mountains along riversides and wet places (Ciocârlan, 2000; Tiță, 2008). *Eupatorii cannabini rhizoma cum radicibus* and *Eupatorii folium* contain different active principles: essential oil, bitter sesquiterpene lactones with germacrane structure (eupatolid, eupatoriopicrin), heteroglycans, flavonosides (heterosides of eupatorin, hispidulin, campherol, quercetin), polyacetylenes, stevioside (a diterpene heteroside, 300 times sweeter than sucrose), triterpenoids, polyphenols, resins, benzofurans (cistifolin, euparin, euparone), pyrrolizidine alkaloids (supinidin, lycopsamine, intermedine), simple sugars, organic acids, vitamins, mineral salts. The medicinal products are choleretic-cholagogue, hepatoprotective, diuretic, anti-inflammatory, laxative-purgative, antibiotic, antiviral, immunostimulatory, nematocide, cytotoxic *in vitro* against malignant cells (Ciulei *et al.*, 1993; Istudor, 2001; Valnet, 2003; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Inula helenium* L., elecampane, horse-heal (*Asteraceae*) is a perennial and robust plant, up to 1.5 m tall, which grows in wet meadows and sometimes cultivated (Ciocârlan, 2000; Tiță, 2008). Essential oil (*Inulae aetheroleum*), sesquiterpene lactones, inulin and stigmasterol were isolated from *Inulae radix*. It has antiseptic, antimycotic, vermifuge, antispasmodic, mucolytic, choleretic-cholagogue and diuretic properties for the treatment of catarrhal bronchitis, mucopurulent bronchitis, emphysema, chronic cough, intestinal parasites (Ciulei *et al.*, 1993; Istudor, 2001; Valnet, 2003; Oniga, 2007; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Centaurea cyanus* L., cornflower (*Asteraceae*) is an annual plant growing in cultivated areas or along roadsides. The flowers are grouped in blue globular anthodiums (Ciocârlan, 2000; Tiță, 2008). *Cyani flos* contains bitter principles, flavones, heteroglycans, polyacetylenes, organic acids. For anti-inflammatory, diuretic and hypoglicemiant action the medicinal product is recommended in conjunctivitis, blepharitis, cystitis, nephritis (Ciulei *et al.*, 1993; Istudor, 1998 and 2001; Valnet, 2003; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Arctium lappa* L., burdock (*Asteraceae*) is a biennial species common in steppe

and ruderal places. In the second year, the stem is branched, pubescent, and can reach up to 2 m tall (Ciocârlan, 2000; Tiță, 2008). *Bardanae radix* contains large quantities of inulin. It has antimicrobial, choleretic, laxative, diuretic, diaphoretic, bitter tonic and cytostatic properties. It is recommended for skin, oral or hepatobiliary disorders, urinary infections and diabetes mellitus (Ciulei *et al.*, 1993; Istudor, 2001; Valnet, 2003; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Cichorium intybus* L., chicory (*Asteraceae*) is a perennial herb of 40–100 cm tall, frequent in steppe zones, ruderal places and crops. Solitary or grouped anthodiums, with terminal or axial disposition, consist of blue ligulate, hermaphrodite flowers (Ciocârlan, 2000; Tiță, 2008). *Cichorii herba et radix* contains mainly bitter principles being used for stomachic, bitter tonic, cholagogue, diuretic, depurative, hypoglycemic and antibacterial action in the treatment of anorexia, urinary disorders, bloating, flatulence, slow digestion, diabetes, constipation (Ciulei *et al.*, 1993; Istudor, 2001; Oniga, 2007; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

*Xanthium spinosum* L., spiny cocklebur, prickly burweed (*Asteraceae*) is an annual weed of 30–50 cm tall, growing in ruderal areas, fields, pastures, roadsides, from plain to mountain. The stem is branched, erect. The leaves are alternate, petiolate, and three-lobed, with the central lobe much longer than the other two, shiny dark green and hairy on the upper surface and downy beneath. About 2–3 cm long yellow spines are found in leaf axils and at stem nodes (Ciocârlan, 2000; Tiță, 2008). Flavones, tannin, saponosides, essential oil, sesquiterpene lactones, phytosterols, carotenoids, polyphenolic acids, sugars, vitamins, organic acids and mineral salts were previously isolated from *Xanthii spinosi herba*. The vegetal product has anti-inflammatory, decongestant and diuretic effect, being recommended for prostate adenoma (Petcu *et al.*, 1980; Kiss and Racz, 1988; Tămaș and Toader, 1989; Ciulei *et al.*, 1993; Istudor, 2001; Tiță *et al.*, 2011b).

*Elymus repens* (L.) Gould. sin. *Agropyron repens* (L.) Beauv., couch grass (*Poaceae*) is a perennial species frequent in steppe areas, cultivated and ruderal places (Ciocârlan, 2000; Tiță, 2008). *Graminis rhizoma* contains polyfructosans (triticin, inulin), mucilages, essential oil (agropyrene, carvone), potassium salts, vitamins (B complex). It has diuretic, depurative, diaphoretic, anti-inflammatory and hypotensive properties in the complex formula of diuretic and diaphoretic teas. In addition, it is recommended for the treatment of digestive and genitourinary inflammations (Ciulei *et al.*, 1993; Istudor, 1998; Valnet, 2003; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

∗ *Zea mays* L., maize (*Poaceae*) is a cultivated species (Ciocârlan, 2000; Tiță, 2008). Due to the content of flavonosides, saponosides, allantoin, stigmasterol, vitamins (C, B6, E, K) the medicinal product *Maydis stigma* has diuretic and soothing action being used in cystitis, metritis and renal calculi (Ciulei *et al.*, 1993; Valnet, 2003; Istudor, 2005; Bojor and Popescu, 2009; Bruneton, 2009; Mencinicopschi *et al.*, 2009).

## CONCLUSIONS

The medicinal plants with diuretic action from Oltenia flora reported in the paper are natural sources of vegetal products used for the treatment of cardiovascular (heart failure, hypertension), urinary and metabolic (obesity, gout) disorders.

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# CONTENT IN METAL CATIONS OF SOME VEGETAL PRODUCTS WITH DIURETIC PROPERTIES FROM OLTENIA FLORA

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***Key words*:** *vegetal products, diuretic properties, metal cations, GF–AAS, microwave digestion*

## ABSTRACT

*Zn, Cr, Cd, Pb, Cu and Fe cations were determined for Eupatorii cannabini folium, Eupatorii cannabini rhizoma cum radicibus, Fraxini folium, Ononidis radix, Origani herba and Xanthii spinosi herba. Samples were analyzed by graphite furnace atomic absorption spectrometry (GF–AAS) with microwave digestion. The studied vegetal products contain all these metals within the safe limits. The results were discussed with respect to quality control of these medicinal plants.*

## INTRODUCTION

Many heavy metals are essential as important constituents of pigments and enzymes, mainly zinc and iron. However, all metals are toxic at high concentration (Ybañez and Montoro, 1996). Because of this, it is necessary to determine these metals in plants used in phytotherapy (Başgel and Erdemoğlu, 2006).

Zinc is a trace mineral necessary within certain limits for a good functioning of the human body, *i.e.* because is the co-factor of superoxide dismutase involved in oxidative defense processes. Zinc excess leads to inhibition of mutagenic cell apoptosis (Steponėnienė *et al.*, 2003).

Copper participates in numerous physiological processes and is an essential cofactor of metal proteins and yet, when there is an excess of copper important cellular processes are affected such as photosynthetic electron transport (Yruela, 2005).

Iron is also a necessary micronutrient in the process of photosynthesis and cofactor of several enzymes whose deficiency leads to chlorosis and necrosis (Breet *et al.*, 2005).

Of these considerations, the control of the heavy metals contents in medicinal and aromatic plants represents worldwide quality control.

The medicinal products *Eupatorii cannabini folium* and *Eupatorii cannabini*

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*rhizoma cum radicibus* contain: volatile oil, bitter sesquiterpene lactones with germacrane structure (eupatolid, eupatoriopicrin), heteroglycans, flavonosides (heterosides of eupatorin, hispidulin, campherol, quercetin), polyacetylene compounds, a sweetening diterpene heteroside (stevioside), 300 sweeten than sucrose, triterpenoids, polyphenols, resins, benzofurans (citifolin, euparin, euparone), pyrrolizidine alkaloids (supinidin, lycopsamine, intermedine), simple carbohydrates, organic acids, vitamins, minerals.

The above-mentioned vegetal products have the following actions: hepato-protective, diuretic, anti-inflammatory, laxative-purgative, antibiotic, antiviral, immuno-stimulatory, nematocide, cytotoxic *in vitro* against malignant cells. They are used to hepatobiliary diseases, acute gastritis, inflammation of the bladder, inflammations and skin, irritations (Ciulei *et al.*, 1993; Istudor, 2001; Stănescu *et al.*, 2002; Bojor and Popescu, 2009; Gîrd *et al.*, 2005; Mencinicopschi *et al.*, 2009).

*Xanthii spinosi herba* contains: flavones, saponosides, tannin, essential oil, sesquiterpene lactones, phytosterols, carotenoids, polyphenolic acids (caffeic acid, chlorogenic acid), sugars, vitamins, organic acids, mineral salts (Kiss and Rácz, 1988; Istudor, 2001; Gîrd *et al.*, 2009). It has an anti-inflammatory, decongestant and diuretic action (Tămaş and Toader, 1989). In the form of pharmaceutical preparations (Proprin, Votis, Prostatin) it is used in the treatment of prostate adenoma (Petcu *et al.*, 1980; Ciulei *et al.*, 1993; Istudor, 2001; Gîrd *et al.*, 2005).

The medicinal product *Fraxini folium* contains: coumarin derivatives (fraxoside, fraxetol, isofraxetol), flavonosides (rutin), catechic tannin, pentacyclic triterpenic acids (ursolic acid), mannitol, inositol, glucose, organic acids, volatile oil, gums. It has diuretic, diaphoretic, laxative, analgesic, anti-inflammatory and uricosuric properties, used in the treatment of gout, arthritis, arthrosis, rheumatism, lithiasis, into the formula of laxative purgative powder and rheumatic tea (Ciulei *et al.*, 1993; Istudor, 1998; Valnet, 2003; Tiță, 2005; Hanganu *et al.*, 2006; Gîrd et al., 2009; Mencinicopschi *et al.*, 2009).

*Ononidis radix* contains mainly triterpenoid saponosides ($\alpha$- and $\beta$-onocerine) and isoflavones. It has diuretic action, facilitates removal of kidney stones, sodium and chloride. It is used to treat kidney lithiasis in the composition of diuretic teas (Nistreanu, 2001; Tiță, 2005; Oniga, 2007; Gîrd *et al.*, 2009; Mencinicopschi *et al.*, 2009).

The medicinal product *Origani herba* contains: essential oil, tannin, flavones, anthocyanins, pentacyclic triterpene acids, sugars, vitamins, organic acids, mineral salts. It has antiseptic, diuretic and spasmolytic actions, being used in the treatment of respiratory and digestive infections, of spastic enterocolites and nervousness, in the form of industrial products (Sedocalm) or teas (sedative, antibronchitis) (Ciulei *et al.*, 1993; Istudor, 2001; Bruneton, 2009; Gîrd *et al.*, 2009).

The aim of these study was to determinate the inorganic profile of *Eupatorii cannabini folium*, *Eupatorii cannabini rhizoma cum radicibus*, *Fraxini folium*, *Ononidis radix*, *Origani herba* and *Xanthii spinosi herba* by graphite furnace atomic absorption spectrometry (GF–AAS) with microwave digestion, a very precise and sensitive method.

## MATERIAL AND METHODS

### Plant material

The vegetal products were harvested before the full flowering period, when the active compounds reach the maximum level. They were taken from different parts of the plant (top, middle or base) both from sunny and shady places.

*Eupatorii cannabini folium* and *Eupatorii cannabini rhizoma cum radicibus* were harvested from *Eupatorium cannabinum* species, hemp agrimony, *Asteraceae* (*Compositae*)

family, located nearby Seaca de Câmp village, Dolj County.

*Xanthii spinosi herba* was collected from *Xanthium spinosum* species, spiny cocklebur, prickly burweed, *Asteraceae* (*Compositae*) family, located nearby Seaca de Câmp village, Dolj County.

*Fraxini folium* was obtained from *Fraxinus excelsior* species, *Oleaceae* family, in Zăval Forest, Dolj County.

*Ononidis radix* was harvested from *Ononis spinosa* species, *Fabaceae* (*Leguminosae*) family, on meadows near Seaca de Câmp village, Dolj County.

*Origani herba* was collected from *Origanum vulgare* species, oregano, *Lamiaceae* family, near Tismana city, Gorj County.

**Sample preparation**

The vegetal products were air dried in a dark place, at constant humidity until constant mass. Plant tissue samples were reduced to 0.5 to 1.0 mm particle size to ensure homogeneity and 0.25 g sample were weighted with accuracy of ±0.0001 g (Kalra, 1998).

**Microwave digestion**

For the determination of the accumulation of metal cations in samples, aliquots (0.5 g) of the dried and powdered biomass were weighed into teflon crucibles and decomposed in 5 mL concentrated nitric acid of high purity and 1 mL 30% hydrogen peroxide.

Digestion was made in a MLS1200 Mega Milestone microwave equipment using a program in five stages: $t_1$=2 minutes at 250 W, $t_2$=2 minutes at 0 W, $t_3$=6 minutes at 250 W, $t_4$=5 minutes at 400 W and $t_5$=5 minutes at 600 W.

A blank was prepared under the same conditions using the same operating mode and the same reagents but replacing the sample with deionized water.

The solutions were transferred quantitatively to measuring flasks and filled up to 100 mL with deionized water.

**Method and apparatus**

Analyses were performed on novAA 400G–Analytik Jena atomic absorption spectrometer with heated graphite furnace and autosampler MPE60 and WinAAS 3.17.0 software.

Working solutions (with concentrations ranging between 0–100 µL) of examined ions were prepared by dilution of standard certified solutions (1000 mg/L).

Working parameters were optimized, calibration standards concentrations were established, an amount of matrix modifier was added [$NH_4H_2PO_4$ and $Mg(NO_3)_2$] in all samples (5 µL). All chemical reagents were of high purity.

## RESULTS AND DISCUSSION

Medicinal plants require a careful monitoring of heavy metal content, even of the elements considered essential knowing that above certain concentrations they are potentially toxic to man.

Atomic absorption spectrometric method provided a wide range of advantages such as: specificity, high sensibility, simple sample preparation, the use of small sample volumes. Sensitivity was calculated as the metal concentration (µg/L) which gives 1% absorption of transmitted radiation (Table 1).

Of the determined metals, Zn, Fe and Cu are essential elements while Cd, Cr

and Pb are non-essential elements, potentially toxic for humans due to their capacity to accumulate in organism. High levels of heavy metals can cause morphological cell abnormalities and mutagenic effects in humans (Kumar *et al.*, 2005).

Amongst vegetal samples, significantly higher were the contents of Fe in *Xanthii spinosi herba* (598.74 μg/g), *Eupatorii cannabini folium* (145.66 μg/g) and *Eupatorii cannabini rhizoma cum radicibus* (200.23 μg/g) (Table 2).

Table 1

Performance parameters obtained by GF–AAS

| Parameters | Metal cations | | | | | |
|---|---|---|---|---|---|---|
| | Cd | Pb | Zn | Cu | Cr | Fe |
| $R^2$ | 0.9989 | 0.9949 | 0.9193 | 0.9987 | 0.9981 | 0.9842 |
| Slope | 0.0954 | 0.0027 | 0.0335 | 0.0108 | 0.0209 | 1.0217 |
| Sensibility [μg/L] | 0.0456 | 1.601 | 0.130 | 0.4012 | 0.2079 | 4.27 |
| Optimum concentration range [μg/L] | 0.91–9.12 | 32.02–320 | 2.6–26 | 8.02–80.24 | 4.15–41.58 | 84–840 |
| Wavelength [nm] | 228.8 | 283.3 | 213.9 | 327.4 | 357.9 | 302.1 |

Table 2

Element concentrations in analyzed samples

| No. | Sample | Concentration of metal cations | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cd [μg/g] | Cu [μg/g] | Cr [μg/g] | Zn [μg/g] | Pb [μg/g] | Fe [μg/g] |
| 1. | Origani herba | – | 12.60 | 10.41 | 15.54 | 2.37 | 84.23 |
| 2. | Ononidis radix | – | 6.34 | 16.28 | 24.47 | 2.95 | 60.82 |
| 3. | Fraxini folium | – | 7.84 | 12.80 | 18.93 | 1.87 | 74.52 |
| 4. | Eupatorii cannabini folium | – | 9.42 | 11.26 | 22.16 | 3.51 | 145.66 |
| 5. | Eupatorii cannabini rhizoma cum radicibus | – | 7.81 | 10.43 | 20.12 | 2.29 | 200.23 |
| 6. | Xanthii spinosi herba | 21.24 | 8.07 | 0.56 | 32.52 | 5.43 | 598.74 |

The level of Cu determined in *Origani herba* was 12.60 μg/g, the highest amount within all samples. In addition, in *Ononidis radix* the maximum levels of Zn and Cr were detected.

The transfer of Cu, Zn is controlled by soil–plant barrier while Cd, Cr and Pb pass from the soil land locate in the veins and petiole of plants. Approximately half of the Pb and Zn ingested by humans come from vegetal food (Dudka and Miller, 1999).

All metals are found in normal limits, but all samples contain $Pb^{2+}$ and $Cr^{3+}$, soil and air pollution markers. Slightly elevated levels of lead in plant tissues could be explained by place of collection of samples located relatively close to intense traffic areas and where from fuel come into the atmosphere tetra alkyl derivatives of this element.

Cadmium instead was not found only in *Xanthii spinosi herba*.

In *Eupatorii cannabini rhizoma cum radicibus*, the heavy metals are in slightly smaller amounts than in *Eupatorii cannabini herba*.

## CONCLUSIONS

Using graphite furnace atomic absorption spectrometry with microwave digestion, metal cations (Zn, Cr, Cd, Pb, Cu and Fe) were determined for the reason of quality control of some vegetal products with diuretic properties from Oltenia flora. The content of metal cations was in safe limits even for Pb and Cr, markers for soil and air pollution.

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# POPULATION STRUCTURE OF THE *LEPIDOPTERA NOCTUIDAE* PESTS FROM THE CABBAGE CROP IN GRADINARI (OLT) AREA

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***Key words*:** cabbage, pests, *Lepidoptera*, *Noctuidae*

## ABSTRACT

*The group of Noctuidae found in cabbage crops from vegetable basin Gradinari is represented by genres Mamestra Ochsen. and Autographa which manifests as dominant and specific pests.The population structure of lepidopteran Noctuidae pests from one year to another, manifest great variety in terms of the participation of each species in the composition of the local population, between the various species associated with the same ecosystem there is a constant struggle for occupation and expanding area. In 2009, the local population was given the dominance of Mamestra brassicae L., with a rate of 64.13% and in 2010, the dominant species is Mamestra oleracea L. with a higher participation rate than the previous year, respectively 44.75%*

## INTRODUCTION

In our country cabbage has a high prevalence area and found favorable growing conditions in all counties, especially on meadows, excepting mountains and arid area (Mitrea, I. 2005). The largest area cultivated in irrigated system are found in the Danube Plain, West Plain, Southeast and the Transylvanian Plateau.

White cabbage is one of the most important vegetable crops which occupy large areas and the pests that attack this crop are numerous and very harmful. Lately there has been a strong multiplication of the pest species and there has increased the damage produced by the cabbage moth (*Mamestra brassicae* L.), turnip flea beetle (*Phyllotreta undulata* Kutsch.), cabbage aphid (*Brevicoryne brassicae* L) and cabbage fly (*Delia brassicae* L.) (Andow, D.A, et al. 1986).

The group of lepidopteran belonging to the *Noctuidae* family, popularly known as moth is represented in our country by a large number of genera and species, most of them are pests for many crops, especially cruciferous crops (Kononenko, V. et al. 1988).

These pests are polyphagous, their larvae feed with aboveground parts of plants in night and morning hours. During the day caterpillars are hiden under the leaves and in the aboveground plant parts near to the soil surface. Damage is visible on leaves and flowers of vegetables and occasionally also on fruits of horticultural plants (Devetak, M. et al 2010).

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Pests prefer specially grounds where weeds are grown or no herbicides are used (Sannino, 2005).

Beside the mechanical damages, caterpillars lessen the quality of crops also through their excrements on flowers and leaves (Pelosini, 1999). Their indirect influence can be observed through the transmission of pathogenic fungi and bacteria into attacked plants (Corvi, 1998).

Surveillance of these pests is absolutely necessary for the preparation of forecast and warning programs, to implement timely control treatments.

## MATERIAL AND METHODS

The research has been made during 2009-2010, at a vegetable farm from Gradinari, Olt County, on an area of 5000 m2, cultivated with different cabbage varieties: early cabbage crop (Dittmark,), summer cabbage (Gloria) and autumn cabbage (de Buzau). The research focused on the harmful entomofauna from the cabbage crop, more accurate on the species from the *Noctuidae* Family, *Lepidoptera* order.

The crop has been set up in the first decade of April. Biological material was collected from cabbage crops as well from the spontaneous flora.

Regarding the collection and identification of the Noctuidae species from the cabbage crop, there were made determinations consisting in:

collecting with entomological net;

collecting with pheromonal traps.

Collecting of biological material was made every ten days, after that the material collected was analyzed and determined with the binocular magnifier glass using the Identification Manual (Godeanu, 2002).

## RESULTS AND DISCUSSIONS

Analyzing seasonal dynamics of the *Lepidoptera Noctuidae* complex in cabbage crops, it appears that the butterflies are present in *Brassicaceae* vegetable crops throughout the vegetation season.

Between different species cohabiting in the same biotope, there is not consistency between development cycles, a phenomenon caused by multiple species of lepidopteran entering the structure of *Mamestra* Ochsen genre., each of which is adapted to the specific requirements of existence.

The group of *Noctuidae* found in cabbage crops from vegetable basin Gradinari is represented by genres *Mamestra* Ochsen. and *Autographa* which manifests as dominant and specific pests.

Unlike like the *Autographa* genre that is represented by a single species - *Autographa gamma* L., *Mamestra* Ochsen. genre is represented locally by a great diversity of species such as: *Mamestra brassicae* L., *Mamestra* (*Discestra*) *trifolii* Rott., *Mamestra oleracea* L., *Mamestra suasa* Den. Et Schiff., *Mamestra dysodea* Den. Et Schiff., *Mamestra blenna* Hb.-G., *Mamestra cappa* Hb.

The population structure of lepidopteran *Noctuidae* pests from one year to another, manifest great variety in terms of the participation of each species in the composition of the local population, between the various species associated with the same ecosystem there is a constant struggle for occupation and expanding area.

Thus, in 2009, the local population was given the dominance of *Mamestra brassicae* L., with a rate of 64.13% and a low contribution of species *Mamestra oleracea* L.

21,30%, *Mamestra* (*Discestra*) *trifolii* Rott. 7,45%, *Mamestra suasa* den. Et Schiff. 4,15% and *Autographa gamma* L. 2,97% (Figure 1).

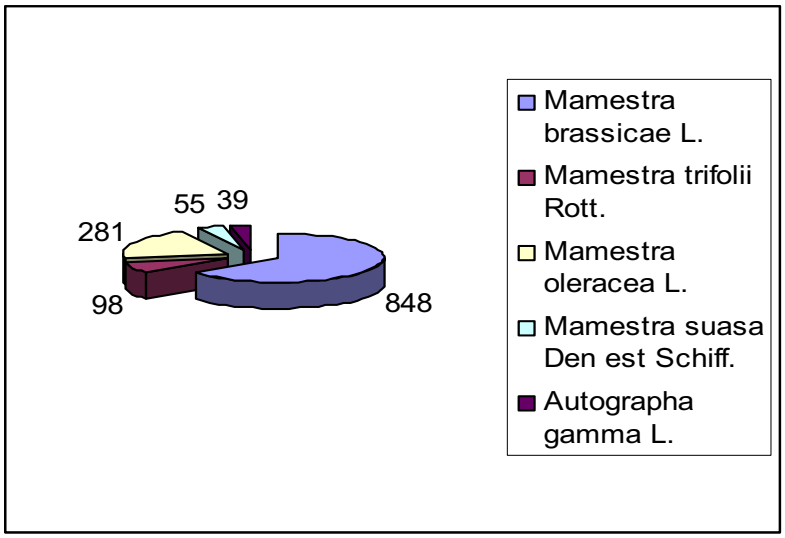


Figure 1.The population structure of lepidopteran *Noctuidae* from cabbage crop in 2009

In 2010, the dominant species are *Mamestra oleracea* L. with a higher participation rate than the previous year, respectively 44.75% and *Mamestra* (*Discestra*) *trifolii* Rott., species with a much higher percentage than in 2009 respectively by 35.44%, the rest of lepidopteran species maintained at values between 3,75% -12,30% (Figure 2).

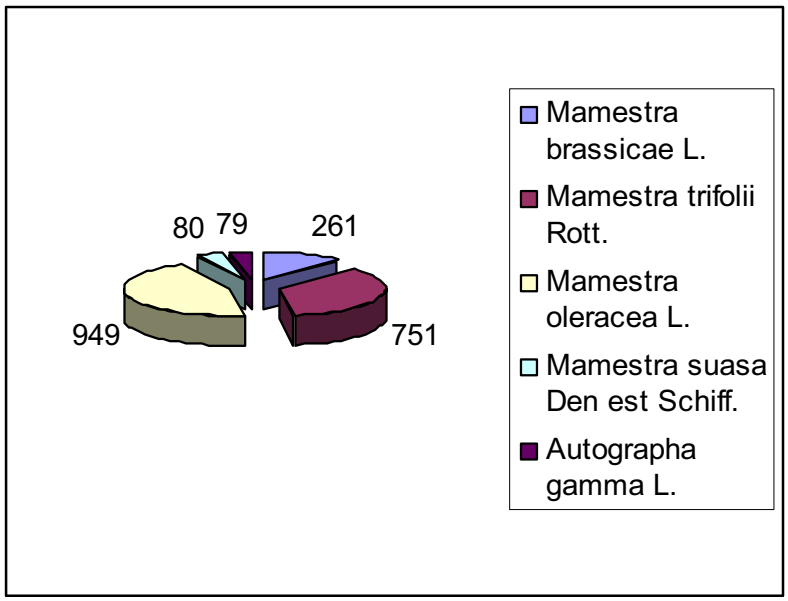


Figure 2.The population structure of lepidopteran *Noctuidae* from cabbage crop in 2010

Also during this period, it appears that in this area have appeared *Noctuidae* new species, such as *Mamestra dysodea* Den. Et Schiff., *Mamestra blenna* Hb. G. *Mamestra cappa* Hb., which under favorable conditions may become potential pests of cabbage.

It can be seen that the structure of *Noctuidae* populations from the cabbage crops recorded from year to year variations in composition and density, which justifies their continuous surveillance in order to draw up proper forecasting and warning schemes.

To explain these variations in structure and dynamics were taken into account: air temperature, rainfall, relative humidity and the winter climatic conditions.

One of the main causes of variations from one year to another, of the structure and number density of adults, is represented by the climatic conditions during the insects hibernating, harsh winters, with high temperature amplitudes in the absence of a protective snow cover and late springs, cool and rainy.

## CONCLUSIONS

Regarding the population structure of lepidopteran Noctuidae from cabbage crop, in Gradinari area, we can conclude that:

- in 2009, the local population was given the dominance of Mamestra brassicae L., with a rate of 64.13%:

- in 2010, the dominant species is Mamestra oleracea L. with a rate of 44.75%.

During 2009 - 2010, in this area have appeared Noctuidae new species, such as Mamestra dysodea Den. Et Schiff., Mamestra blenna Hb. G. Mamestra cappa Hb., which under favorable conditions may become potential pests of cabbage.

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# STUDIES ON THE COLEOPTERAN PESTS FROM THE FRUIT-TREES ECOSYSTEMS S. D. BANU MARACINE CRAIOVA

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***Key words***: *pests, entomofauna, coleoptera, fruit-growing ecosystem*

## ABSTRACT

*The human intervention on the fruit-trees ecosystems had a major influence, therefore these showed a great imbalance and a low complexity because of the human activity. Despite the frequent pesticide application, there are resistant pests that continue to frequently attack these ecosystems.*

*For a better knowledge of these pests, the study of the entomofauna of coleopteran pests was necessary during the year 2011. After the carried out collections, numerous species of coleoptera were identified and systematically classified in 4 families.*

*The most numerous species within the 4 families were Curculionidae, Cetoniidae, Rutilidae and Rhynchitidae.*

## INTRODUCTION

From the total number of animal species inhabiting the Earth, the insects represent 70%. Every year the entomology experts discover and describe new species and according the latest information from literature, approximately 2 million species of insects are known in the world nowadays.

Having a high ecological plasticity (phenotypic plasticity), the coleoptera are found in all ecosystems – from the polar regions to the equatorial forests. They are common in all kind of habitats, they feed on any kind of food.

The impact of the pests on the cultures is the result of the interaction between a certain host/parasite system and the local environmental and crop conditions. Due to the expansion of the ecological agriculture, there were modifications of the spectrum of diseases and pests from the crops.

Taking in consideration the position and the role of the coleoptera from different types of ecosystems, the current study was necessary in this type of fruit-trees ecosystems. (Mitrea I., 2005)

Numerically the coleoptera are superior to other groups of beetles, being in most ecosystems more than 80% of all insect species existing.

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The coleoptera meet various useful functions for humans, are good indicators can serve as one of the main objects solving problems and integrated environmental monitoring.

In the presence of many ecosystems and their frequency could be constructed mathematical models of the dynamics of various animals of economic interest, developed principles and ways of forecasting and control various dangerous pests in agriculture and forestry.

Identify pests in fruit growing ecosystem attack various organs of vegetation, following a summary we found that the most affected organs of vegetation: aerial organs of plants, floral organs majortatea attacking adults, buds or shoots, some attack and fruits. Larvae that attack fruits and they are generally harmful, consuming mesocarp and seeds (Perju T., B. Bobirnac, D Bob, 1976), (Rosca I, Oltean I., Mitrea I., şi colab. 2011).

## MATERIALS AND METHODS

In order to correctly establish the entomofauna of the coleopteran pests from S. D. Banu Maracine Craiova, during the year 2011, collecting was done by different methods: Barber traps method, striking method, etc. , (Chimisliu Cornelia. 2001)

The most helpful method was the use of Barber traps, which were put at ground level. The collected entomofauna was analyzed and identified within the laboratory by using a magnifying glass and microscope (figure 1, 2).

The identification was done by using the public Romanian fauna identifier (Panin, 1951). The resulting material was labeled and preserved in ethylic alcohol 70 % and glycerin or it was prepared on dry needles (figure 3).

Figure 1. Barber Trap

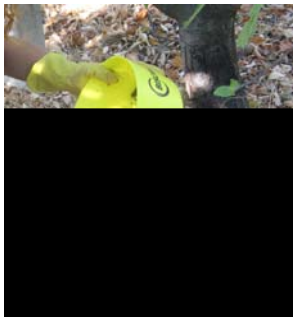

Figure 2. Barber Trap

Figure 3. Material set on entomological needles

## RESULTS AND DISCUSSION

After the collecting done in the year 2011, the harmful entomofauna from the fruit-trees ecosystem S. D. Banu Maracine Craiova was identified. The collected coleoptera were submitted to a statistical analysis regarding their abundance and they were grouped in species and families.

Within the fruit-trees ecosystem from the S. D. "Banu Maracine" there were also identified 4 coleoptera families summing a number of 6 species, represented by 40 collected individuals (table 1).

Table no. 3

The abundance of coleopteran species from the fruit-trees ecosystem from the S. D. "Banu Maracine"

| Nr. Crt | Fam. / Species | Individual no. |
|---|---|---|
| | Fam. Curculuionidae | |
| 1. | Anthonomus pomorum | 9 |
| 2. | Rhynchites bacchus | 4 |
| 3. | Sciaphobus squalidus | 5 |
| | Fam. Cetoniidae | |
| 4. | Oxythirea funesta | 7 |
| | Fam. Rutilidae | |
| 5. | Epicometis hirta | 7 |
| | Fam. Rhynchitidae | |
| 6. | Rhynchites aequatus | 8 |

The most numerous family is represented by *Curculuionidae* with 3 distinct species, *Anthonomus pomorum* with 9 individuals, *Rhynchites bacchus* with 4 individuals and *Sciaphobus squalidus* with 5 individuals.

The next 3 families had only a single representative as follows: *Fam. Cetoniidae* with *Oxythirea funesta* presented a number of 7 individuals, *Fam. Rutilidae* with the species *Epicometis hirta* with 7 individuals and *Fam. Rhynchitidae* with *Rhynchites aequatus* with 8 individuals. (Figure 4)

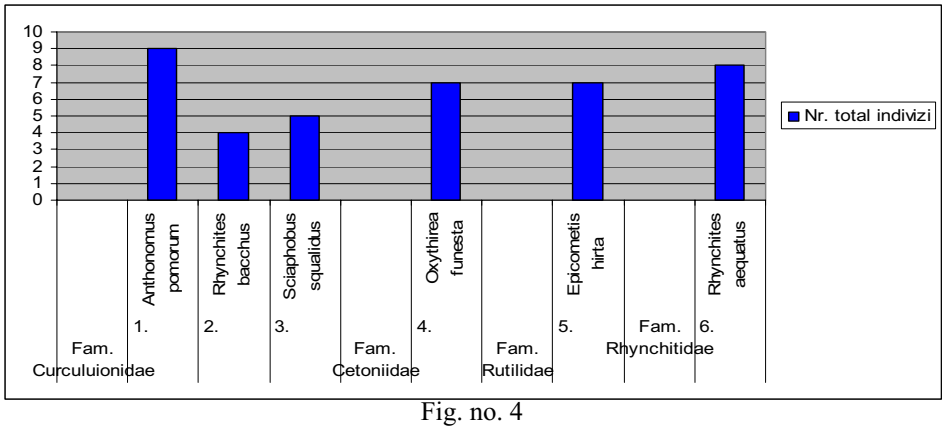


Fig. no. 4

The abundance of coleopteran species from the fruit-trees ecosystems

The total number of coleoptera identified in the fruit-trees ecosystem was 40 individuals.

## CONCLUSIONS

The identified entomofauna was represented by 6 species which belong to 4 different families. From the total number of individuals the most abundant species was *Anthonomus pomorum* with a total number of 9 individuals, followed by *Rhynchites aequatus* with 8 individuals. The smallest number of individuals was represented by *Rhynchites bacchus* with only 4 individuals.

## ACKNOWLEDGMENT

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# ASSESSMENT OF HETEROSIS FOR SPIKE LENGTH IN WINTER BARLEY

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***Keywords***: heterosis, spike length, barley.

## ABSTRACT

*Spike length is one of the important components of yield and this is the source of assimilates closer to the caryopses. The choice of parental material used in the hybridization scheme does contribute significantly for the development of a suitable genotype. The present studies were under taken to estimate the level of heterosis and heterobeltiosis for spike length among 15 $F_1$ hybrids of six winter barley varieties, with different genetic and ecological origin. Most of the hybrids exhibited remarkable heterosis over mid and better parents for the spike length with average values of 17.94 and 12.03 %. The hybrids GK Metal x Victoria, GK Judy x Gunda and GK Judy x Victoria which showed the highest mid and better parent heterosis values for was the best combination which can be considered for selection of pure line varieties. The high values of heterosis and of potency ratio for spike length proved that the nature of inheritance for this trait was over dominance.*

## INTRODUCTION

Spike length is one of the important components of yield and this is the source of assimilates closer to the caryopses. Spike structure has more benefits of utilizing illumination than the other parts of the plant. It will also stay green and functional for a longer time together with the awns. Because of these features, it contributes, on the average, with 20–30% of the dry matter accumulated in the kernels. The exploitation of genetic information has not been very well demonstrated for this vital yield contributing trait in barley (Slafer et al. 2002). The choice of plant breeding methodology for upgrading the yield potential largely depends on the availability of reliable information on the nature and magnitude of gene effects present in the population (Bos and Caligari, 1995).

The present studies were under taken to estimate the level of heterosis and heterobeltiosis for spike length among 15 $F_1$ hybrids of six winter barley varieties, with different genetic and ecological origin.

## MATERIAL AND METHOD

The biological material comprised of 15 hybrids resulting from a diallel cross between six winter barley varieties (Andra, Vitoria, GK Metal, GK Judy, Gunda and Malwinta) with different ecological and genetic origin. The research was conducted based on a randomized complete block design with three replications. At maturity, from every

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plot, 20 plants of $F_1$'s and their parents were randomly selected and carried out measurements of spike length.

The values of mid parent heterosis and better parent heterosis (heterobeltiosis) were estimated as formulated by Matzinger et al., (1962) and Fonseca & Patterson (1968). Heterosis % = ($F_1$-MP) x 100/ MP ; Heterobeltiosis = ($F_1$- BP) x 100/BP, where $F_1$, MP and BP denote the performance of hybrid, average performance of parents and performance of better parent, respectively.

Inheritance nature was evaluated based on dominance parameters. The potency ratio was computed by ratio of dominance parameters (***d/a***), where *d* is the difference between $F_1$ means and parent means, and *a* is the half difference of two parents for a combination, according to the formula: $d/a = (F_1 - MP) / ½(P_1 - P_2)$.

To estimate significant differences among parents and $F_1$ hybrids, the data were subjected to statistical analysis by using the analysis of variance technique (Ciulca, 2006). Significant differences were further subjected to Multiple Range Test. The 't' test was manifested to determine whether $F_1$ hybrid means were statistically different from mid parent and better parent means (Wynne et al., 1970).

## RESULTS AND DISCUSSIONS

Most of hybrids (73.3%) showed a significantly higher spike length to both parental forms, while 26.7% of hybrids have registered significantly higher values of this trait than one of the parents.

The studied hybrid combinations (table 1) showed an amplitude of 2.46 cm for this trait, with limits ranging from 9.84 cm at Andra x Gunda hybrid to 12.30 cm in GK Metal x Victoria. The highest values of this trait and also significant increases were recorded in the crosses: GK Metal x Victoria, GK Judy x Gunda, Malwinta x Gunda, GK Judy x Victoria, Malwinta x GK Judy. Significantly lower values of spike length were recorded for the hybrids: Andra x Gunda, Andra x Victoria, GK Metal x Andra.

Table 1.

Average values of spike length in the studied $F_1$ hybrids

| Hybrids | Average (cm) $\bar{x} \pm s_{\bar{x}}$ | Difference to $P_1$ | Difference to $P_2$ |
|---|---|---|---|
| Malwinta x Victoria | 11.00±0.27bcde | 0.29 | 1.55*** |
| Malwinta x Andra | 10.60±0.15cde | -0.11 | 2.27*** |
| Malwinta x Gunda | 11.89±0.21ab | 1.18** | 1.93*** |
| Malwinta x GK Judy | 11.76±0.14abc | 1.05** | 2.14*** |
| Malwinta x GK Metal | 11.28±0.23abcd | 0.57 | 2.47*** |
| GK Judy x Victoria | 11.86±0.23ab | 2.24*** | 2.41*** |
| GK Judy x Andra | 10.64±0.16cde | 1.02*** | 2.31*** |
| GK Judy x Gunda | 12.06±0.20ab | 2.44*** | 2.10*** |
| GK Judy x GK Metal | 10.96±0.25bcde | 1.34*** | 2.15*** |
| GK Metal x Victoria | 12.30±0.19a | 3.49*** | 2.85*** |
| GK Metal x Andra | 10.42±0.23de | 1.61*** | 2.09*** |
| GK Metal x Gunda | 11.28±0.27abcd | 2.47*** | 1.32** |
| Andra x Victoria | 10.36±0.26de | 2.03*** | 0.91* |
| Andra x Gunda | 9.84±0.23e | 1.51*** | -0.12 |
| Victoria x Gunda | 11.32±0.27abc | 1.87*** | 1.36** |
| *LSD 5%* | *1.19cm* | | |

The mid parent heterosis showed positive values and high variability with the limits between 7.60 % for Andra x Gunda and 34.72 % for GK Metal x Victoria hybrid. In all studied combinations values for that heterosis were statistically ensured. The GK Metal x Victoria hybrid had a significantly higher value of mid parent heterosis to the other combinations except GK Judy x Victoria. Also in the case of combinations: GK Judy x Victoria, GK Judy x Gunda, GK Metal x Andra and GK Metal x Gunda, high values for over 20 % of heterosis were observed.

The heterobeltiosis showed generally positive values except for the combinations Malwinta x Andra and Andra x Gunda. So, this heterosis for spike length of individual hybrids ranged from – 1.,20 % at Andra x Gunda to 30.16 % for GK Metal x Victoria with an overall average of 12.03 %. Values higher than 20 % and significantly increases of heterobeltiosis were highlighted by the hybrids: GK Metal x Victoria, GK Judy x Victoria and GK Judy x Gunda.

Table2.

Heterosis and potency ratio values for spike length in $F_1$ hybrids of six winter barley varieties

| Hybrids | MP | | BP | | PR |
|---|---|---|---|---|---|
| | H (%) | HDev (cm) | H (%) | HDev (cm) | |
| Malwinta x Victoria | de 9.13* | 0.92cd | de 2.71 | 0.29cd | 1.46 |
| Malwinta x Andra | cde 11.34*** | 1.08cd | e -1.03 | -0.11d | 0.91 |
| Malwinta x Gunda | bcde 15.05*** | 1.56bcd | bcde 11.02** | 1.18bcd | 4.15 |
| Malwinta x GK Judy | bcde 15.69*** | 1.60bcd | bcde 9.80** | 1.05bcd | 2.93 |
| Malwinta x GK Metal | bcde 15.57*** | 1.52bcd | cde 5.32 | 0.57cd | 1.60 |
| GK Judy x Victoria | ab 24.38*** | 2.33ab | ab 23.28*** | 2.24ab | 27.35 |
| GK Judy x Andra | bcde 18.55*** | 1.67bcd | bcde 10.60*** | 1.02bcd | 2.58 |
| GK Judy x Gunda | b 23.19*** | 2.27ab | ab 21.08*** | 2.10ab | 13.35 |
| GK Judy x GK Metal | bcd 18.94*** | 1.75bc | bcd 13.93*** | 1.34bc | 4.31 |
| GK Metal x Victoria | a 34.72*** | 3.17a | a 30.16*** | 2.85a | 9.91 |
| GK Metal x Andra | bc 21.59*** | 1.85bc | abc 18.27*** | 1.61abc | 7.71 |
| GK Metal x Gunda | bcd 20.19*** | 1.90bc | bcde 13.25** | 1.32bc | 3.30 |
| Andra x Victoria | bcde 16.54*** | 1.47bcd | bcde 9.63** | 0.91bcd | 2.63 |
| Andra x Gunda | e 7.60* | 0.70d | e -1.20 | -0.12d | 0.85 |
| Victoria x Gunda | bcde 16.64*** | 1.62b | bcd 13.65*** | 1.36bc | 6.33 |
| *LSD 5%* | *11.14* | *1.01cm* | *14,66* | *1,39cm* | *d/a=5.96* |

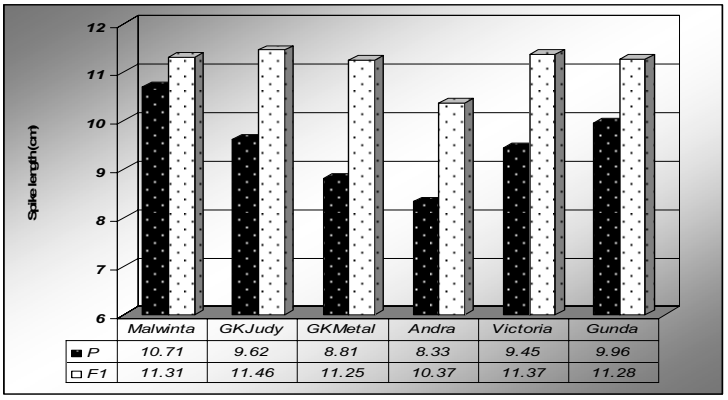
Heterosis deviations to mid-parents were significantly higher in GK Metal x Victoria, GK Judy x Victoria and GK Judy x Gunda Greiff. To the best parent, the highest heterosis deviations were recorded in GK Metal x Victoria, GK Judy x Victoria and GK Judy x Gunda. For most of studied hybrid combinations (except Malwinta x Victoria, Malwinta x Victoria, Malwinta x GK Metal şi Andra x Gunda) significant values of heterobeltiosis have been registered.

Table 3

Express manner of the spike length in $F_1$ hybrids

| Hybrids number | Proportion and number of $F_1$ hybrids | | | | Range towards parents mean(%) | Mean heterosis (%) | |
|---|---|---|---|---|---|---|---|
| | Higher towards parents | Between parents | | Lower towards parents | | "MP" | "BP" |
| | | Upper mean | Under mean | | | | |
| 15 | 13 (86.6 %) | 2 (13.4 %) | - | - | 7.60 – 34.72 | 17.94 | 12.03 |

According to the values of potency ratio the nature of inheritance for this trait was over dominance for most hybrid combinations with direction towards higher parent, and partial dominance in Malwinta x Andra and Andra x Gunda. The greatest contribution of non additive gene effects in the inheritance of this trait were observed in hybrids: GK Judy x Victoria and GK Judy x Gunda




| | Malwinta | GKJudy | GKMetal | Andra | Victoria | Gunda |
|---|---|---|---|---|---|---|
| P | 10.71 | 9.62 | 8.81 | 8.33 | 9.45 | 9.96 |
| F1 | 11.31 | 11.46 | 11.25 | 10.37 | 11.37 | 11.28 |

Fig. 1. Spike length for groups of $F_1$ hybrids with the same recurrent parent

Regarding the groups of hybrids with the same recurrent parent (fig. 1) there is an amplitude of 1.09 cm with limits between 10.37 in the hybrids of Andra variety, while the hybrids of GK Judy variety showed the highest value of spike length.

## CONCLUSIONS

The hybrids GK Metal x Victoria, GK Judy x Gunda and GK Judy x Victoria which showed the highest mid and better parent heterosis values for was the best combinations which can be considered for selection of pure line varieties

The high values of heterosis and potency ratio for spike length proved that the nature of inheritance for this trait was over dominance, with a significant role of non additive genetic variance.

The study also provides a basis for the selection of better parents which might prove to be good combiners and hence could be used in the breeding program to develop barley varieties with higher values for spike length

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**Vol. XVII ( LIII ) - 2012**

# ASSESSMENT OF HETEROSIS FOR GRAIN YIELD PER SPIKE IN WINTER BARLEY

Varzaru Simona[1], Ciulca S.[2]

***Key words***: heterosis, spike yield, barley.

## ABSTRACT

*Grain yield per spike is a complex trait made up of the interaction between different spike components and environmental effects. These characters are polygenic and exhibit additive and non-additive genetic variation. The present studies were under taken to estimate the level of heterosis and heterobeltiosis for grain yield per spike among 15 $F_1$ hybrids of six winter barley varieties, with different genetic and ecological origin. Most of the hybrids exhibited remarkable heterosis over mid and better parents for the spike yield with average values of 23.59 and 16.44 %. The hybrids Malwinta x Victoria, Malwinta x Andra and Malwinta x GK Judy which showed the highest mid and better parent heterosis values for was the best combination which can be considered for selection of pure line varieties. To utilize non-additive gene effects which were higher in magnitude, breeding methods involving reciprocal recurrent selection or biparental mating were suggested for further improvement in grain yield per spike.*

## INTRODUCTION

Grain weight is an essential component contributing for yield production, so positive heterosis is desirable for this trait. An understanding of the genetic factors that govern the yield components is necessary because breeding for yield depends largely upon genetic manipulation of the components along with yield.

The choice of selection and breeding procedures for genetic improvement of any crop is largely depends on the knowledge of type and relative amount of genetic component and the presence of non-allelic inter-action for different characters in the plant materials under investigations (Goldringer et al. 1997).

According to Morgan et al. (1989), if parents show high yielding potential, heterosis for grain yield would be less because parents have already many beneficial genes in homozygous state. In addition, Fabrizius et al., (1998) reported that the more genetic differences among parents are, the more heterosis can be possible positively for grain yield in a hybrid. Also, Singh et al., (2004), suggested that especially heterosis over better parent (heterobeltiosis) can be useful for determining true heterotic cross combinations. In fact, heterosis shows combining ability of parents so their usefulness in hybridization programs.

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## MATERIAL AND METHOD

The biological material comprised of 15 hybrids resulting from a diallel cross between six winter barley varieties (Andra, Vitoria, GK Metal, GK Judy, Gunda and Malwinta) with different ecological and genetic origin. The research was conducted based on a randomized complete block design with three replications. At maturity, from every plot, 20 plants of $F_1$'s and their parents were randomly selected and carried out measurements for grain weight per spike.The values of mid parent heterosis and better parent heterosis (heterobeltiosis) were estimated as formulated by Matzinger et al., (1962) and Fonseca & Patterson (1968). Heterosis % = ($F_1$-MP x 100)/ MP; Heterobeltiosis = ($F_1$-BP x 100)/BP, where $F_1$, MP and BP denote the performance of hybrid, average performance of parents and performance of better parent, respectively.Inheritance nature was evaluated based on dominance parameters. The potency ratio was computed by ratio of dominance parameters (d/a), where d is the difference between F1 means and parent means, and a is the half difference of two parents for a combination, according to the formula: d/a = (F1 - MP) / ½(P1 - P2).To estimate significant differences among parents and $F_1$ hybrids, the data were subjected to statistical analysis by using the analysis of variance technique (Ciulca, 2006). Significant differences were further subjected to Multiple Range Test. The't' test was manifested to determine whether $F_1$ hybrid means were statistically different from mid parent and better parent means (Wynne et al., 1970).

## RESULTS AND DISCUSSIONS

Approximately 47 % of hybrid combinations showed a grain weight per spike lower than 2 g, while 33 % of the hybrids showed a spike yield between 2 and 2.2 g, in the case of 20 % of the hybrids the grain weight per spike was over 2.2 g. The highest values of this trait, over 2.3 g, and statistically ensured differences were reported for the combinations: Malwinta x GK Judy and Malwinta x Victoria. A grain weight per spike above 2 g was also achieved by the two hybrids of Malwinta variety and four hybrids of GK Judy variety. The smaller values of spike yield were recorded in three hybrids of Andra variety, namely: Andra x Gunda, Andra x Victoria and GK Metal x Andra. Most of hybrids (73.3 %) showed a significantly higher spike yield to both parental forms, while 26.7 % of hybrids have registered significantly higher values of this trait only towards one parent

Table1.

Average values of spike yield in the studied $F_1$ hybrids

| Hybrids | Average (cm) | Difference to $P_1$ | Difference to $P_2$ |
|---|---|---|---|
| Malwinta x Victoria | 2.32±0.07ab | 0.53*** | 0.76*** |
| Malwinta x Andra | 2.20±0.05abc | 0.41*** | 0.81*** |
| Malwinta x Gunda | 2.20±0.08abc | 0.41*** | 0.44*** |
| Malwinta x GK Judy | 2.42±0.08a | 0.63*** | 0.58*** |
| Malwinta x GK Metal | 1.98±0.08cdef | 0.19 | 0.35** |
| GK Judy x Victoria | 2.04±0.08bcde | 0.20* | 0.48*** |
| GK Judy x Andra | 2.06±0.06bcde | 0.22** | 0.67*** |
| GK Judy x Gunda | 2.24±0.08abc | 0.40*** | 0.48*** |
| GK Judy x GK Metal | 2.17±0.06abcd | 0.33*** | 0.54*** |
| GK Metal x Victoria | 1.99±0.06cde | 0.36*** | 0.43*** |
| GK Metal x Andra | 1.86±0.05def | 0.23** | 0.47*** |
| GK Metal x Gunda | 1.92±0.05cdef | 0.29*** | 0.16* |
| Andra x Victoria | 1.82±0.07ef | 0.43*** | 0.26** |
| Andra x Gunda | 1.65±0.10f | 0.26* | -0.11 |
| Victoria x Gunda | 1.95±0.06cdef | 0.39*** | 0.19* |
| *LSD 5%* | *0.34 g* | | |

The mid parent heterosis showed positive values and high variability with the limits between 4.76 % for Andra x Gunda and 38.51 % for Malwinta x Victoria hybrid. For most hybrid combinations the values of that heterosis were statistically ensured. The Malwinta x Victoria and Malwinta x Andra hybrids have submitted a significantly higher value of mid parent heterosis to the hybrids: Andra x Gunda, GK Metal x Gunda, Malwinta x GK Metal, Victoria x Gunda and GK Judy x Andra. Also in the case of combinations: GK Judy x GK Metal, GK Judy x Andra and GK Metal x Gunda, high values of heterosis for over 25 % were observed.

Table2.

Heterosis and potency ratio values for spike yield in $F_1$ hybrids of six winter barley varieties

| Hybrids | MP | | BP | | PR |
|---|---|---|---|---|---|
| | H (%) | HDev (cm) | H (%) | HDev (cm) | |
| Malwinta x Victoria | a38.51*** | 0.65a | ab 29.61*** | 0.53ab | 5.61 |
| Malwinta x Andra | a38.36*** | 0.61a | abc 22.91*** | 0.41abc | 3.05 |
| Malwinta x Gunda | abc23.94*** | 0.43ab | abc 22.91*** | 0.41abc | 28.33 |
| Malwinta x GK Judy | ab33.33*** | 0.61a | a 31.52*** | 0.58a | 24.20 |
| Malwinta x GK Metal | cd15.79** | 0.27bc | c 10.61 | 0.19c | 3.38 |
| GK Judy x Victoria | bcd20.00* | 0.34b | c 10.87* | 0.20c | 2.43 |
| GK Judy x Andra | abc27.55*** | 0.45ab | c 11.96** | 0.22c | 1.98 |
| GK Judy x Gunda | abc24.44*** | 0.44ab | abc 21.74*** | 0.40abc | 11.00 |
| GK Judy x GK Metal | abc25.07*** | 0.44ab | abc 17.93*** | 0.33abc | 4.14 |
| GK Metal x Victoria | abc24.76*** | 0.40ab | abc 22.09*** | 0.36abc | 11.29 |
| GK Metal x Andra | abc23.18*** | 0.35b | bc 14.11** | 0.23c | 2.92 |
| GK Metal x Gunda | cd13.27*** | 0.23bc | c d9.09* | 0.16cd | 3.46 |
| Andra x Victoria | abc23.39** | 0.35b | abc 16.67** | 0.26bc | 4.06 |
| Andra x Gunda | d4.76 | 0.08c | d -6.25 | -0.11d | 0.41 |
| Victoria x Gunda | bcd17.47*** | 0.29bc | c 10.80* | 0.19c | 2.90 |
| *LSD 5%* | *16.93* | *0.26g* | *15.72* | *0.28g* | *d/a=7.28* |

The heterobeltiosis showed generally positive values except for the combination Andra x Gunda. So, this heterosis for spike yield of individual hybrids ranged from – 6.25 % at Andra x Gunda to 31.52 % for Malwinta x GK Judy with an overall average of 16,44 %. The highest values and significantly increases of heterobeltiosis were highlighted by the hybrids: Malwinta x GK Judy and Malwinta x Victoria. Heterosis deviations to mid-parents were significantly higher in Malwinta x Victoria, Malwinta x Andra and Malwinta x GK Judy. To the best parent, the highest heterosis deviations were recorded in Malwinta x GK Judy and Malwinta x Victoria. According to the values of potency ratio the nature of inheritance for this trait was over dominance for most hybrid combinations with direction towards higher parent, and partial dominance in Andra x Gunda. The greatest contribution of non additive gene effects in the inheritance of this trait were observed in hybrids: Malwinta x Gunda and Malwinta x GK Judy. For Andra x Gunda the low value of potency ratio which not exceeded the half unity proved that the inheritance of these traits is controlled especially by the additive gene action.

Table 3

Express manner of the spike yield in $F_1$ hybrids

| Hybrids number | Proportion and number of $F_1$ hybrids | | | | Range towards parents mean(%) | Mean heterosis (%) | |
|---|---|---|---|---|---|---|---|
| | Higher towards parents | Between parents | | Lower towards parents | | "MP" | "BP" |
| | | Upper mean | Under mean | | | | |
| 15 | 14 (93.3 %) | 1 (6.7 %) | - | - | 4.76 – 38.51 | 23.59 | 16.44 |

Regarding the groups of hybrids with the same recurrent parent (fig. 1) it is noted that the lowest value of grain weight per spike was performed by the hybrids of Andra variety, while the hybrids of Malwinta variety showed the highest value of this trait.

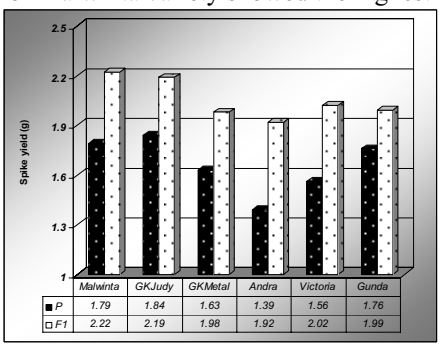


Fig. 1. Spike yield for groups of $F_1$ hybrids with the same recurrent parent

Compared to recurrent parent, the highest differences are seen in the groups of hybrids where this parental form (Andra and Victoria) submit lower values of grain weight per spike. The hybrids group of Gunda variety show the lowest values of both heterosis.

## CONCLUSIONS

Most of the hybrids exhibited remarkable heterosis over mid and better parents for the spike yield with average values of 23.59 and 16.44 %.

The hybrids Malwinta x Victoria Malwinta x Andra and Malwinta x GK Judy which showed the highest mid and better parent heterosis values for was the best combination which can be considered for selection of pure line varieties.

To utilize non-additive gene effects which were higher in magnitude, breeding methods involving reciprocal recurrent selection or biparental mating were suggested for further improvement in grain yield per spike.

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# STUDIES CONCERNING THE EFFECTS OF OSMOTIC STRESS ON SEED GERMINATION AND EARLY EMBRYO GROWTH IN SORGHUM [Sorghum bicolor (L.) Moench]

Viliga F[1]., Sumalan R[1].

**KEYWORDS**: *seed, germination, embryo growth, osmotic stress, sorghum bicolor.*

## ABSTRACT

*Romania is one of the European countries with vast areas of low productivity soils containing toxic salts, being frequently associated with poverty. The problems determined by osmotic stress are associated mostly with other abiotic stress factors, as drought and phosphorus deficit. The effects of osmotic stress on seed germination and early embryo growth were investigated in laboratory in three cultivars of sorghum – cv. "Jumbo", cv. „Sugargraze I" and „Sugargraze II"– to verify how stress may limit crop growth during the very early stages of growing season. Two water potentials (ψ) of the germination solution (from 0 to-0,3 and -0,6 MPa) in polyethylene glycol (PEG-6000) for osmotic stress tests were studied. Daily germination was recorded, radicle and shoot lengths were measured during 7 days after initial germination. Seed germination was reduced in stress conditions, and shoot/ root growth was adversely affected. Cv. „Jumbo" was less sensitive to both stresses.*

## INTRODUCTION

Due to their sedentary mode of life, plants resort to many adaptive strategies in response to different abiotic stresses such as high salt, dehydration, cold, heat and excessive osmotic pressure, which ultimately affect plant growth and productivity (Prabhjot et al., 2002). Among the stages of the plant life cycle, seed germination is one of the most important key processes in the survival and growth of plants (H. Siti Aishah et al. 2002). Seed germination is usually the most critical factor determining success or failure of plant establishment (M. Jami Al-Ahmadi and M. Kafi , 2006). A better understanding of how seeds germinate under osmotic stress, germination percentage, rate of germination, germination index and the coefficient of velocity of germination will allow a better knowledge and the development of new varieties that can have a higher yield in these situations (Kader, M.A. and S.C. Jutzi, 2004, Bayuelo-Jimenez et al., 2002).

## MATERIAL AND METHODS

The experiment was conducted in the Plant Physiology Laboratory of Faculty of Horticulture and Forestry at Banat University of Agricultural Sciences and Veterinary Medicine of Timisoara, Romania.

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The germination experiments were carried out in January 2012. The seeds were incubated at laboratory conditions at 25°C and were monitored until the end of the germination period of 7 days. Seeds of the three sorghum cultivars namely, cv. "Jumbo", cv. „Sugargraze I" and „Sugargraze II". Seeds were surface sterilized with NaOCl 5% for 7 minutes, then rinsed three times with sterilized distilled water. To hurry the germination, after sterilization the seeds were stored in sterilized distilled water for six hours at normal light and room temperature. The seeds were germinated in covered, sterilized, disposable Petri dishes lined with filter paper and moistened with distilled water (control), or polyethylene glycol (PEG-6000) solution (from 0 to-0,3 and -0,6 MPa). Three replicates of 100 seeds were used for each treatment and cultivar. Every second day, 5 mL of the respective treatments were applied to each Petri dish and to maintain adequate moisture. This experiment was arranged in a Completely Randomized Design (CRD). There were 3 replicates per treatment. Every day biometrical measurements were estimated, namely daily germination, radicle and shoot lengths were measured during 7 days after initial germination.Germination rate was calculated using the following formula:

$\sum GT_1/T_1 + \ldots. + GT_n/T_n$

where, GT is seeds germinated each day and T refers to the day during the trial. The Germination Index (GI) was calculated based on the following formula:

GI = (5xn1) + (4xn2) + … + (1xn5)

where, n1, n2, n3, n4 and n5 are the number of seeds germinated on days 5, 4, 3, 2 and 1, respectively. The Coefficient of Velocity of Germination (CVG) was estimated from the formula:

CVG = (N1 + N2 + • • • + Nx)/100 x (N1T1 + • • • + NxTx)

Where:

N = No. of seeds germinated each day

T = No. of days from seeding corresponding to N. (Kader and Jutzi, 2004).

Average and standard deviation were calculated for each treatment.

## RESULTS

The highest germination rate was observed at cultivar Jumbo, even at the highest PEG concentrations, meanwhile Startgraze II was the most affected by the increase of osmotic stress. Considering the germination speed, the highest mean values at V0 were registered at Sugargraze II cultivar, but in the same time this was seriously affected by the increase of PEG concentration. The highest germination speed at -0.6 MPa was at Jumbo cultivar.Germination index presents very high variability amongst the cultivars and PEG concentrations. Thus Jumbo cultivar has an average germination index at Vo and also at V1 variants above 600, and only at V2 variant, the values decrease below 500. The Sugargraze cultivar has slightly lower values of germination index than Jumbo, but the mean values vary similarly. Instead, at Sugargraze II we could observe a more drastic decrease of germination index, from 575,33 at V0 variant, to 244 at the highest PEG concentration of V2. Very similar to this index is the variation of coefficient velocity of germination (Table 1). It can be concluded that there is a very high variability amongst the three sorghum cultivars regarding their salt tolerance in germination phase.

We could observe a very high variability of coleoptil and radical development under different PEG concentrations at the three sorghum cultivars. After 7 days of development, the cultivar with the longest radicle at V0 was Sugargraze II, but at the highest osmotic stress cultivar Jumbo developed the longest radicles. The coleoptil was even more affected by the osmotic stress. The cultivar with the longest coleoptils at the highest PEG concentration was Sugargraze II (Table 2).

Table 1.

Characteristics of germination for sorghum cultivars.

| Variants | Germination (%) | Germination speed | Germination index | Coefficient velocity of germination |
|---|---|---|---|---|
| Jumbo | | | | |
| V0 | 96.666 ± 2.08 | 130.47 ± 2.12 | 643.666 ± 14.36 | 78.722 ± 3.27 |
| V1 | 95 ± 1.00 | 119.7 ± 1.26 | 636.666 ± 12.22 | 80.499 ± 2.56 |
| V2 | 84.666 ± 2.08 | 99.93 ± 5.35 | 562 ± 13.75 | 68.999 ± 3.21 |
| Sugargraze I | | | | |
| V0 | 88.333 ± 4.16 | 107.94 ± 10.70 | 584.666 ± 27.06 | 72,305 ± 2.65 |
| V1 | 86 ± 3.00 | 105.37 ± 10.67 | 565.666 ± 16.16 | 69.222 ± 1.99 |
| V2 | 64.666 ± 0.58 | 60.57 ± 1.04 | 423.666 ± 5.13 | 50.833 ± 0.93 |
| Sugargraze II | | | | |
| V0 | 90.666 ± 2.52 | 135.99 ± 4.55 | 575.333 ± 21.03 | 65.916 ± 3.53 |
| V1 | 76.666 ± 6.43 | 98.51 ± 20.12 | 485.666 ± 36.75 | 54.722 ± 3.10 |
| V2 | 38.333 ± 9.71 | 24.98 ± 12.13 | 244 ± 62.96 | 27.944 ± 7.28 |

## CONCLUSION

The speed and successfulness of germination and also, the growth of radicle and coleoptil of embryo is highly affected by the osmotic stress. To be cultivated in saline soils, it is important for sorghum cultivars to germinate and develop even if in soil is a high osmotic stress, thus is crucial to make a selection of cultivars to find the proper genotype for every environmental conditions. Under osmotic stress conditions. the cultivar with the best germination (germination rate, speed, index and coefficient velocity of germination) is Jumbo, followed by Stargraze I and Stargraze II. After germination, the growth of embryo is also affected by PEG solution with simulates the osmotic stress. The cultivars which developed the best under higher PEG conditions are Jumbo and Sugargraze I.

## ACKNOWLEDGEMENT

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Table 2.
The effect of osmotic stress on the radicle and coleoptil growth, mean and standard deviation. V0 - control, V1 - PEG -0.3 MPa, V2 - -0.6 MPa

| Number of days | Jumbo | | | Sugargraze I | | | Sugargraze II | | |
|---|---|---|---|---|---|---|---|---|---|
| | V0 | V1 | V2 | V0 | V1 | V2 | V0 | V1 | V2 |
| Radicle | (mm) | | | | | | | | |
| 1 | 11.8±0.12 | 8.23±0.21 | 5.77±0.32 | 8.93±0.91 | 5.53±0.06 | 3.4±0.1 | 4.9±0.53 | 5.5±0.4 | 2.97±0.12 |
| 2 | 24.4±0.35 | 17±0.53 | 11.9±0.75 | 18.4±1.62 | 11.7±0.4 | 7.33±0.21 | 10.4±1.18 | 11.5±0.6 | 6.47±0.23 |
| 3 | 35.2±1.26 | 22.2±1.91 | 15.4±0.57 | 31.3±2.25 | 20.5±1.78 | 10.4±0.76 | 18.2±2.4 | 17.4±1.99 | 7.93±0.31 |
| 4 | 43.3±2.7 | 31.8±3.1 | 20.2±0.62 | 37.7±3.85 | 28.7±0.81 | 16.8±0.62 | 28.3±2.86 | 22.2±2.27 | 11.1±0.91 |
| 5 | 50.4±0.2 | 39.9±4.95 | 23.3±2.16 | 55.6±3.1 | 34.5±2.77 | 18±0.7 | 29.5±2.76 | 26.3±1.88 | 11.3±0.87 |
| 6 | 52.1±0.17 | 41.1±5.11 | 23.9±2.55 | 57.7±2.93 | 36±3.18 | 18.7±0.51 | 30.5±3.16 | 27.4±2.06 | 11.6±0.95 |
| 7 | 52.8±0.51 | 42±5.3 | 24.4±2.35 | 62.4±3.92 | 37.9±2.89 | 19.8±0.32 | 32.2±2.76 | 28.5±2.09 | 12.1±1.11 |
| Coleoptil | | | | | | | | | |
| 1 | 3.07±0.15 | 1.53±0.35 | 0.07±0.12 | 2.43±0.29 | 0.5±0.3 | 0.00±0.00 | 1.84±0.32 | 1.38±0.2 | 0.03±0.06 |
| 2 | 6.67±0.15 | 3.5±0.56 | 0.23±0.25 | 5.27±0.42 | 1.5±0.53 | 0.07±0.06 | 4.43±0.42 | 3.1±0.26 | 0.33±0.06 |
| 3 | 15.1±1.07 | 6.5±0.36 | 0.5±0.36 | 10.5±0.75 | 3.4±0.56 | 0.43±0.25 | 8.3±1.41 | 5.27±0.5 | 0.53±0.06 |
| 4 | 26.5±4.27 | 9.67±0.91 | 0.93±0.65 | 18.9±2.36 | 6.37±1.14 | 0.97±0.49 | 15.9±0.83 | 6.43±0.46 | 0.73±0.06 |
| 5 | 38±4.1 | 11.2±0.4 | 1.23±0.57 | 27.6±2.78 | 7.03±1.35 | 1.13±0.55 | 25.8±1.66 | 7.73±0.4 | 0.8±0.1 |
| 6 | 46.6±2.12 | 11.7±0.56 | 1.33±0.57 | 29.8±2.26 | 8.07±1.35 | 1.63±0.4 | 36.7±2.15 | 9.3±0.61 | 0.87±0.15 |
| 7 | 53±2.82 | 13.2±0.95 | 1.4±0.7 | 42.3±4.9 | 10.1±1.7 | 2.33±0.42 | 41.3±2.86 | 10.5±0.85 | 0.9±0.17 |

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# THE PATHOLOGICAL ASPECTS OF THE DOG URINARY SYSTEM CAUSED BY THE UROLITHIASIS

Carmen Vladulescu[1], Safta D.[2]

***Key words****: urolithiasis, calculi, carbonates, excretion.*

## ABSTRACT

*The urate are the most common in Dalmatians dogs and dogs with portosistemic congenital vascular. The formation of urine and other factors. Dalmatians fail to convert their urate in allantoin metabolism, due to their liver is ammonium urate calculi depends on the concentration of ammonium urate and absolute normal.*

*After hand feelings and ultrasound examination of the abdomen it has been only emphasized the bladder which presents stones. In order to analyses the chemical structure of calculus in bladder the following surgical conduct was performed: surgery performed in a medical cabinet-vet; to the bladder was found a number of 9 calculus; calculus were sent to the laboratory to analyze their chemical composition.*

*After the lab analyses the structure of calculus showed: carbonate, at a rate of about 83% and urate, at a rate of about 17%.*

## INTRODUCTION

Urolithiasis or presence of stones in the urinary tract (bladder, urethra) is considered as the main cause of high protein dog food. The stones are usually composed of phosphates, carbonates, ammonium urate or calcium oxalate.

The most common urinary stones are composed of struvite in dogs. The mineral composition is most struvite, but there are also present small amounts of carbon and ammonium urate. In most cases struvite calculi are formed in association with urinary tract infections, caused by bacteria of the genus Staphylococcus spp. and Proteus spp.

Urinary injections must be treated: choosing the antibiotics must take into consideration the sensibility of bacteria. Most infections with Staphylococcus and Proteus respond to treatment with amoxicillin and ampicillin.

The urate are the most common in Dalmatians dogs and dogs with portosistemic congenital vascular. The formation of urine and other factors. Dalmatians fail to convert their urate in allantoin metabolism, due to their liver is ammonium urate calculi depends on the concentration of ammonium urate and absolute normal. Liver cells of these dogs cannot absorb uric acid and hence, the transformation of uric acid to allantoin is not where to

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Dalmatians which were fed a diet rich in animal protein excrete a concentrated combination of ammonium and urate; so increases the risk of stone formation of ammonium urate. Alcalinization of urine reduces the ammoniac production of the kidney, the objective is to realize a urinary pH > 7. If necessary the alcalinization of urine can be achieved by administering NaHCO3, 1 g (1/4 little spoon)/5kg, three times a day with food. Citrates potassium, administered in this regard (25-50 mg/kg/day) is a tastier alternative. Fresh water should also be provided all the time.

The symptoms are similar to cystitis: common urinate from which result just a few drops of red urine or nearly normal urine.

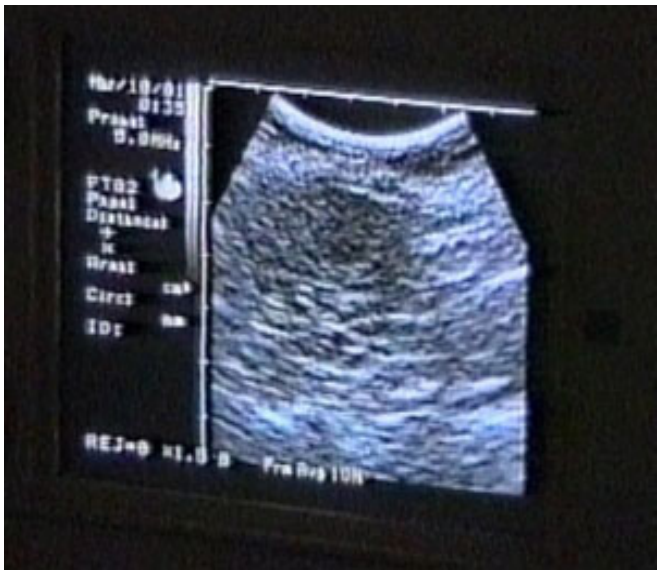
## MATERIAL AND METHOD

A female dalmatian dog, aged 10 years old came to the medical cabinet presenting a voluminous in the lower abdomen, bleeding while urinating, increase in weight and difficulty in urinating. After hand feelings and ultrasound examination of the abdomen it has been only emphasized the bladder which presents stones.

To diagnose, the following were done:

- clinical consulting, signs of the disease being obvious since the entry of dogs in medical cabinet
- ecography or computed tomography (CT)

As method of treatment it was proceeded to extract the calculus out of the bladder, which were identified when the ecography of the abdomen was performed. In picture 1 it can be seen the ecography where the calculus can be identified.



Picture 1. Calculus in the urinary bladder

In order to analyses the chemical structure of calculus in bladder the following surgical conduct was performed:
- surgery performed in a medical cabinet-vet
- to the bladder was found a number of 9 calculus
- calculus were sent to the laboratory to analyze their chemical composition.

## RESULTS AND DISCUSSIONS

Urination calculus usually consist of several types of minerals, the most frequent being those that contain struvit or calcium oxalate. It is essential to know their composition because the therapeutic approach is completely different, depending on their type. Laboratory analysis is the one that tells us what type of minerals contain calculus, or if they contain certain combinations of minerals.

After the lab analyses the structure of calculus showed:
- carbonate, at a rate of about 83%
- urate, at a rate of about 17%.

The urinary calculus eliminated after the surgical operation are shown in picture 2. The nine calculus which were eliminated had different sizes.

Picture 2. Calculus eliminated by surgical operation

## CONCLUSIONS

The routine laboratory measurements should include calcium, serum total phosphatase and chloride content. Dissolution of carbonate rocks or calcium oxalate by medical means has not been considered as effective until the present. Treatment involves surgical removal or lithotrities followed by prevention strategies.

It has also been proved that food has a great influence in the process of developing the calculus. The richer in proteins the dog food is, the greater the risk of developing calculus is. It has also been proved that these calculi can also develop in association with the urinary infections produced by Staphylococcus spp. and Proteus spp.

As a method of treatment the chemically destruction of stones is not possible. Therefore, the only method is surgery. After surgery, to prevent a new formation of stones, a dietetic treatment will be given, and as possible low in protein. A supplementary quantity of water should be introduced in the dog's diet in order to increase the diureses and decrease the chemical concentrations.

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# THE INFLUENCE OF STAPHILOCOCCUS IN THE VERTEBRAL MODIFICATIONS CAUSED BY DISKOSPONDYLITIS

Carmen Vladulescu[1], Safta D.[2]

*Key words: diskospondylitis, intervertebral disk, vertebral infection, staphylococcus, pathogenic agents.*

## ABSTRACT

*Diskospondylitis is an infection of the intervertebral disk with concurrent osteomyelitis of adjacent vertebral end plates and vertebral bodies. Infection confined to the vertebral body is referred to as vertebral osteomyelitis. Synonyms for diskospondylitis include intradiskal osteomyelitis, diskitis, intervertebral disk infection, and vertebral spondylitis.*

*Organisms most commonly associated with diskospondylitis are Staphylococcus aureus and S. intermedius. They have been cultured from blood, urine, or both in a significant number of patients with diskospondylitis.*

*Immunosuppressed patients may be predisposed to developing diskospondylitis. Areas of the spine most commonly affected include the lumbosacral junction, cervicothoracic junction, thoracolumbar; junction, and midthoracic disks.*

## INTRODUCTION

Diskospondylitis usually occurs in large purebred dogs, usually weighing between 30-35 kg, with affected males outnumbering females approximately two to one. Large male purebred dogs may have a higher degree of activity and thus place more stress on the spine, presumably predisposing these areas to infection. Some reports suggest that Great Danes and German shepherds are overrepresented. Dogs are generally older than 1 year, and the risk of diskospondylitis increases with age (i.e., 5 to 9 years) (Burkert et al, 2005).

A recent study identified three pathogens that had not been previously reported as causing diskospondylitis: Pseudomonas aeruginosa, Enterococcus faecalis and Staphylococcus epiderinidis (Adamo et al, 2001). Infection generally originates in a nonvertebral location (i.e., urinary tract infection, pyogenic dermatitis, valvular endocarditis, and dental disease) and spreads hematogenously.

Vertebral infection usually begins in the end plate, where sludging blood in sinusoidal veins predisposes to bacterial colonization. Bacteria diffuse through the cartilaginous end plate of the vertebral body to contact the disk, causing lysis of the adjacent end plate, disk necrosis, and collapse of the intervertebral space. Rarely, bacteria migrate dorsally and cause epidural abscess formation. Vertebral physitis, characterized by

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bone lysis initially confined to the caudal physeal zone of the affected vertebra and sparing the vertebral end plates, has been reported in some dogs. Immunosuppressed patients may be predisposed to developing diskospondylitis.

## MATERIAL AND METHOD

Diskospondylitis should be taken into account in patients with sow of spinal cord injury and systemic diseases.

Myelography and/or cross-sectional imaging (computed tomography or magnetic resonance imaging) is mandatory in patients requiring surgical intervention.

Therapy depends on presenting neurologic examination, laboratory data (urine, blood, and/or bone cultures and Brucella titer), and serial neurologic examinations. Generally, patients that are brought in with pain alone or pain and mild paresis are treated with analgesics, antibiotics, and 4 to 6 weeks of strict confinement, regardless of the number of vertebrae affected. Specific antibiotic therapy is based on culture and susceptibility testing of urine, blood, and/or bone and Brucella titer.

In order to prove the postulated in the literature, the following study was done: all large dogs that arrived at medical cabinet were monitored.

To diagnose, the following were done:

- Clinical consulting, signs of the disease being obvious since the entry of dogs in medical cabinet
- X-rays
- computed tomography (CT) or magnetic resonance imaging (MRI)
-

## RESULTS AND DISCUSSIONS

Physical and neurologic examination findings vary, depending on location, severity, and secondary effects of the infection. Patients brought in early in the course of the disease may have systemic signs (i.e., depression, weight loss, and fever); evidence of spinal involvement includes hyperpathia without paresis. Patients brought in later generally have more subtle signs of systemic involvement coupled with more profound neurologic signs (i.e., severe, single or multilevel hyperpathia, with varying degrees of paresis caudal to the lesion).

After monitoring the dogs, the following were observed:

Tabel no.1

Monitoring the large dogs over a period of 4 months

| Period | Number of dogs arrived in the clinic, out of which: | | Dogs having the signs of the disease: | |
|---|---|---|---|---|
| | male | female | male | female |
| February- May | 16 | 13 | 12 | 4 |

Picture 1:Lateral toraco-lombary radiography of a large dog suffering of diskospondylitis

Picture 2: Lateral radiography of a dog with L3-L4 diskospondylitis.
Notice the intradiskal lysis, vertebral end plate lysis, ventral spondylosis, and sclerosis surrounding lysis margins.

Picture 3: Toraco-lombary radiography with signs of diskospondylitis

## CONCLUSION

Diagnosis of diskospondylitis is confirmed by survey radiographs or cross-sectional imaging. The earliest radiographic signs may take 2 to 4 weeks to be visible after initial infection and include lysis of one or both vertebral end plates followed by collapse of the intervertebral disk space. As the infection progresses, radiographic signs of continued vertebral end plate lysis, proliferative bony changes adjacent to the disk space, sclerotic margins, and ventral osseous proliferation with varying degrees of bridging spondylosis deformans result (Fig. 1, 2, 3). Vertebral body lysis followed by vertebral body shortening and spinal instability occurs infrequently, depending upon the bacterias virulence.

The hallmark of diskospondylitis is end plate lysis. Patients with spondylosis deformans (an asymptomatic and generally incidental radiographic finding) do not have end plate lysis.

Antibiotics for use in diskospondylitis: amikacin, clindamicin, doxicycline, minocycline.

Approximately 80% to 90% of patients respond to appropriate medical management. Patients unresponsive after 7 to 10 days of medical therapy should be treated with a different antibiotic. Analgesics are indicated in animals in pain.

Other bacteria and fungi isolated from affected animals are: Staphylococcus aureus, Staphylococcus intermedius, Brucella canis, Streptococcus spp., Escherichia coli, etc.

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# BEHAVIOUR OF SOME ROSES VARIETIES GROWN IN THE BOTANICAL GARDEN CRAIOVA AT THE ATTACK OF SOME HARMFUL AGENTS AND PEST AND THE EFFECTIVENESS OF ALTERNATIVE TREATMENTS

Vulpe M.[1],Mitrea Rodi[2]

***Key words****: roses, pathogen agents, treatments , plant infusions, nettle macerate.*

## ABSTRACT

*Research of some roses varieties grown in the Botanical Garden Craiova at the attack of some pathogen agents and pest, revealed a differently attack degree function the resistance of each variety. During 2012, in order to determine the attack frequency and intensity of the pathogen agents and pests, were performed a total of three treatments using chemicals and plant extracts and infusions.*

## INTRODUCTION

Since ancient times man has felt the need to have beautiful and decorative plants around him, standing testimony to this effect numerous motifs discovered during archaeological excavations.

Perhaps the earliest evidence is a medal discovered in a tomb in the Altai, dated about 7000 years, and that on one side had a rose in relief [Cantor, 2009].

Roses have a long history. Following archaeological discoveries, it was concluded that the rose has existed for over 35 million years. Today there are over 30,000 varieties of roses, this species is the most complex of all flower species [Beales, 2000].

The rose is the most popular ornamental plant and is considered the "Queen of Flowers." It is cultivated in all countries across five continents. Rose makes its presence felt by the explosion of flavor and color in parks and gardens from spring to late autumn. (Wagner, 2002).

Genus Rosa has 140 species: 95 species are from Asia, 18 species in North America and the rest come from Europe and northwest Africa. The most favorable area for the culture of roses is the northern hemisphere, where the climate is temperate, and in the southern hemisphere hasn't been found any species of rose [Austin, 1998].

Variety varieties and various intermediate forms are very difficult depending clara. Function the delimitation mode of species, these may be in number of 100 according to some specialists or to more than 200 varieties (Eckart Haenchen, 2005). The rose, like any

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plant, is vulnerable to the attack of harmful agents. Affection caused by pests and diseases generally occur in spring. Often an appropriate cultivation is not enough to prevent diseases (Dr. D.G.Hessayon, 2005).

## MATERIAL AND METHOD

Biological material studied was represented by 5 varieties of roses cultivated in the Botanical Garden of the University of Craiova (Table 1). Identifying the attack of the harmful agents took place in the climatic conditions of the year 2012, by performing macroscopic observations on the attacked organs in different vegetation period.

The attack estimation produced by the fungus Sphaerotheca pannosa var rosae and rose Scale (Macrosiphum rosae L.) was performed by establishing the attack frequency (F%), attack intensity (I%). After processing the collected data using the usual formulas, there has been calculated the attack degree (AD%).

Table 1

Varieties studied

| Variety | Description |
|---|---|
| Richard Tauber | Is part of Rose floribunda group. Bush height is up to 70-90cm, the flower is yellow. |
| Nina Weibull | Belongs to floribunda group roses. Bush height is 40-50cm medium with uniform growth and foliage dark green, glossy. Abundant flowers (35 petals), medium-large to large clusters, large whorls red petals. |
| Iceberg | Part of rose floribunda group, high vigor 80-100 cm with displayed branches, few thorns. Flowers semi-abundant, medium grouped in clusters of up to 15, slightly fragrant pure white petals. |
| Criterion | Part of the rose floribunda group, bush heights 70-90 cm, Flower pink, less fragrant. |
| Wettra | Part of Rose theahibrida group, high vigor, bush height 100-120 cm. Leaves are glossy dark green, big flower velvet dark red, unscented. |

In order to set the symptomatically tableau of the harmful agents attack and the evolution dynamics, there has been made macroscopic observations at the studied plants in different vegetation period on different organs, that manifested attack symptoms. In the spring of 2012, at the beginning of the second decade of May, has been observed first symptoms of the attack, produced by Sphaerotheca pannosa var rosae and the rose scale (Macrosiphum rosae L.). The attack estimation has been determined by analysis of 10 shoots on each plant.

To determine the effectiveness of some chemicals comparative with plant extracts there were used following products: Fastac 10 EC, Champ 77 WG, infusion of fresh leaves of horseradish, infusion of nettle.

The nettle macerate has been used as an insecticide for control the rose aphid and has been obtained by macerating 1 kg of nettle harvested when flowering, finely chopped and put to macerate in 5 liters of water for 9 days in the dark (Heinz Erven, 2006).

Horseradish leaf infusion was obtained from 150 g of fresh horseradish leaves, chopped and infused in 5 liters of boiling water and then filtered (Agnes Gedda, 2007). This horseradish infusion has been used as fungicide.

During the vegetation period has been applied 3 treatments, starting with 23rd of May, when has been observed first symptoms of the harmful agents attack.

Variants for the treatments has been the following:

variant 1: FASTAC 10 EC + SHAVIT F 72 WP;

variant 2 : FASTAC 10 EC + CHAMP 77 WG;

variant 3: nettle macerate + horseradish infusion.

## RESULTS AND DISCUSSIONS

As shown in the tables 3 and 4 the rose varieties studied behaved differently at the attack of the fungus Sphaerotheca pannosa var. Rosae (Table 2.) as well at the attack of the rose aphid (Macrosiphum rosae L) (Table 3).

Table 2

Behaviour of some rose varieties at the attack of the fungus *Sphaerotheca pannosa* var. Rosae

| Nr.crt | Variety | F% | I% | AD% |
|---|---|---|---|---|
| 1 | Richard Tauber | 54,4 | 30,07 | 16,88 |
| 2 | Nina Weibull | 79,96 | 64,26 | 51,83 |
| 3 | Iceberg | 21,06 | 7,55 | 4,65 |
| 4 | Criterion | 65,53 | 43,13 | 28,49 |
| 5 | Wettra | 44,43 | 19,92 | 9,1 |

Table 3

Behaviour of some rose varieties at the attck of the rose aphid *Macrosiphum rosae L*

| Nr.crt. | Variety | F% | I% | GA% |
|---|---|---|---|---|
| 1 | Richard Tauber | 41,06 | 17,07 | 18,05 |
| 2 | Nina Weibull | 48,86 | 24,06 | 11,92 |
| 3 | Iceberg | 17,76 | 3,47 | 0,72 |
| 4 | Criterion | 58,86 | 34,83 | 20,70 |
| 5 | Wettra | 35,53 | 12,8 | 4,77 |

Following treatments made to control the powdery mildew attack rose and the rose aphid, there has been recorded the following results shown in Table 4 and Table 5.

Table 4

The efficacy of some fungicides comparative with the horseradish infusion used for controlling the fungus *Sphaerotheca pannosa* var. Rosae

| Variant | Product | Concentration | Efficacy % |
|---|---|---|---|
| 1 | Shavit F 72 WP | 0,2% | 81% |
| 2 | Champ 77 WG | 0,3% | 30% |
| 3 | Horseradish infusion | 150 g horseradish leaves in 5 l water | 65% |

Table 5

Insecticide efficacy Fastac 10 EC versus nettle macerate used to control the rose aphid *Macrosiphum rosae* L

| Variant | Product | Concentration | Efficacy % | |
|---|---|---|---|---|
| | | | 24 h | 120 h |
| 1 | Fastac 10 EC | 0,02 | 82% | 70% |
| 2 | Nettle macerate | 1 kg nettle leaves in 5 l water | 61% | 43% |

## CONCLUSIONS

During the research at the Botanical Garden of the University of Craiova on the behavior of some rose varieties at the attack of the fungus Sphaerotheca pannosa var. Rosae and rose aphid (Macrosiphum rosae L) we can conclude that:

- at the attack of Sphaerotheca pannosa var. Rosae highest resistance has been recorded at the Iceberg variety, at the other end Nina Weibull variety has been the most sensitive variety at the fungus attack.

- regarding the attack of the rose aphid (Macrosiphum rosae L) highest resistance has been recorded at the Iceberg variety while the most sensitive varieties at the rose aphid attack has been Criterion and Richard Tauber.

Regarding the products used for controling *Sphaerotheca pannosa var. Rosae* and *Macrosiphum rosae L* the highest efficacy has been recorded for FASTAC 10 EC (70%) and SHAVIT F 72 WP (81%) comparative with horseradish infusion (65%) and nettle macerate (43%).

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# RESEARCH ON THE CHANGES OF SOME PHYSIOLOGICAL PARAMETERS IN PRUSSIAN CARP (*Carassius auratus gibelio Bloch* 1782) UNDER THE ACTION OF THE LEAD

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Păunescu Alina[3], Marinescu Al. G.[4]

**Key words**: *Prussian carp, heavy metals, lead, oxygen consumption, breathing frequency*.

## ABSTRACT

*In our experiments we followed the effects of sub lethal and lethal concentrations of lead on some physiological parameters of the Prussian carp (Carassius auratus gibelio Bloch 1782). The Pb under the concentrations of 1 mg / l water, 2 mg / l water, 4 mg / l water, had , overall, an inhibitory effect on oxygen consumption for the prussian carp ( Carassius auratus gibelio Bloch 1782).In all the researched concentrations, lead modified the values of breathing frequency for the Prussian carp. The acute test records mortality only in fish variants from the concentrations level of 2 mg Pb / l water, 4 mg Pb / l water, the last variant record absolute mortality.*

## INTRODUCTION

Air, soil, and water pollution is one of the most important problems of this era, affecting human and living organisms and deteriorating natural resources (Khan et al., 2000). Presently, water resources are on the decrease, with a contaminant reduction in water quality. Chemical analysis allows determination of the extent of pollution but not that of its damage to living organisms (Rai et al., 2002). Environmental pollution by toxicants has become one of the most important problems in the world (Chandran et al. 2005).

Metals are non-biodegradable, and once they enter the aquatic environment, bioconcentration may occur in fish tissue by means of metabolic and biosorption processes (Carpene et al., 1990 ). From an environmental point of view, bioconcentration is important because metal ions usually occur in low concentrations in the aquatic environment and subtle physiological effects go unnoticed until gross chronic reactions (e.g. changes in populations' structure, altered reproduction, *etc*.) become apparent.

The heavy metal and pesticide contamination of aquatic system has attracted the attention of researchers all over the world (Dutta & Dalal, 2008) and has increased in the

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last decades due to extensive use of them in agricultural, chemical, and industrial processes that are becoming threats to living organisms. Fish are more frequently exposed to these pollutants because it is believed that regardless of where the pollution occurs, it will eventually end up in the aquatic environment.

Fish have the ability to accumulate heavy metals in their tissue to higher level than the toxic concentration in their environment by absorption along the gill surface and gut, and their respiratory system differs from all other systems because the gills are the main target of pollutants and damage to gills has immediate impact on the rest of the fish body (Al-Yacoob et al., 1994), and human can be at a great risk through contamination of the food chain (Costa & Hartz, 2009). Heavy metals enter fish bodies through three following ways: body surface, gill or the digestive tract (Pourang, 1995). Lead was found to accumulate in fish muscle in some studies (Bradley & Morris, 1986; Wagner & Boman, 2003).

Lead belongs to the group of non-essential and toxic metals, which implies that it has no known function in biochemical processes (Adeyeye et al., 1996).

Further, the toxicity of lead is known to decline with increasing calcium and magnesium concentrations in water. The highest admissible lead concentration in water is 0.004 to 0.008 mg per litre for salmonids and 0.07 mg per litre for cyprinids. (Výzkumný Ustav Rybářský & Svobodová, 1991 ). Because of stability of metals, the organism cannot protect itself with metabolism activities from toxic effects (Farkos et al., 2003).

This study was carried out to analyze the effects of sub lethal and lethal concentrations of lead on some physiological parameters of the Prussian carp (*Carassius auratus gibelio Bloch 1782)*.

## MATERIAL AND METHODS

Determinations were performed on samples of Prussian carp (*Carassius auratus gibelio Bloch1782)* caught in the lakes and rivers bordering Pitesti. Animals were acclimatized for 2 weeks before the completion of experiments in aquariums with a capacity of 100 l and 50 l (Picoş & Năstăsescu, 1988), under conditions of natural photoperiodism, a period in which they were fed once a day (ad libitum), at around 9 a.m. After acclimatization in the laboratory, fish were separated into lots and placed in four experiments. The lead concentrations that have been used have been established by preliminary survival test. The immersion of fish in these solutions has been made after they have been well stirred and aired for five minutes. The water temperature has been between $18^0$ C and $20^0$ C, the immersion solution has been changed every 24 hours (semi static test) and the water has been continuously aired: the fish have been fed during the experiments. The energetic metabolism, expressed by the oxygen consumption, was determined by using the closed respiratory chamber method (the oxygen dose in the water was established by using the Winkler chemical method) (Picoş & Năstăsescu, 1988; Năstăsescu, 1986). These determinations were made at intervals of 24,48,72,96,168 and respectively 336 hours. The breathing frequency was determined at the same intervals as in the case of the energetic metabolism. The selected end-points - the level of glycemia and the number of erythrocytes – were measured with the glucometer (for glycemia) and the Thoma counter chamber (for erythrocytes) (Picoş & Năstăsescu, 1988).

The statistical interpretation of the results was performed with ANOVA test (SPSS 16.0 software for Windows).

## RESULTS AND DISCUSSIONS

In all experimental variants that have been applied on fish, all the stages described by Schäperclaus in the sympthomatologycal scheme for the intoxicated fish (Năstăsescu, 1986, Picoş & Năstăsescu, 1988), have only been observed in the variant with the lead in concentrations 2mg/l water and 4 mg lead /l water, where they have succeeded each other at very short intervals (the fish died in the first 24 experimental hours). The negative effect of heavy metals on fish is related to the disturbance in their biochemical and physiological processes (Villela *et al*., 1999).

The first Figure shows the average frequency of the respiratory movements of Prussian carps exposed to the action of lead at temperature of 18°C - 20°C. For all concentrations, the lead effect of breathing frequency has been strongly inhibiting.

Changes in oxygen consumption of Prussian carps exposed to the lead concentrations in different concentration are shown in Figure 2. Lead had an inhibitory effect on the energy metabolism of Prussian carps. Acute lead intoxication is characterized, first of all, by damage to the gill epithelium: the affected fish are killed by suffocation (Výzkumný Ustav Rybářský & Svobodová ,1991). Goss and Wood (1988) suggested that heavy metals act on gill function resulting in a decrease in oxygen consumption rate because of ion regulatory and acid-base disturbance.

Reduction of oxygen consumption rate in fish exposed to heavy metals indicate the onset of hypoxia under metallic stress (James, 1990), because metals accumulate in gill epithelium and induce lesions like necrosis, thickening and separation of respiratory epithelium (Peuranen *et al*., 1994; Hassan, 2005), also it may resulted in an increase of diffusion distance between the water and blood which makes oxygen absorption difficult.

The main reason of death in fish exposed to heavy metals is the hypoxia because the metals act on the gill function and structure causing damage of the gill epithelia, disturbances in osmo-regulation process, decrease of oxygen consumption and then death (Albaster & Lioyd, 1982; Peuranen *et al*., 1994; Hassan, 2005). Reduction of oxygen consumption rate in fish exposed to heavy metals indicate the onset of hypoxia under metallic stress (James, 1990), because metals accumulate in gill epithelium and induce lesions like necrosis, thickening and separation of respiratory epithelium (Peuranen *et al*., 1994; Hassan, 2005), also it may resulted in an increase of diffusion distance between the water and blood which makes oxygen absorption difficult (Dalzell & MacFurtan, 1994; Aardt & Booysen, 2004). In addition, metals may impair the respiratory surface function by reducing the respiratory surface area through the atrophy and fusion of secondary lamellae, as well as the internal action of metal, which enhances the action of respiratory inhibiting factors. (Muthukumarvel *et al*. 2007; Shereena &Logswamy, 2008).

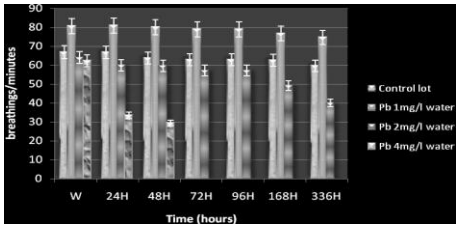


Figure 1.The influence of lead upon breathing frequency on prussian carp (*Carassius auratus gibelio* B.)

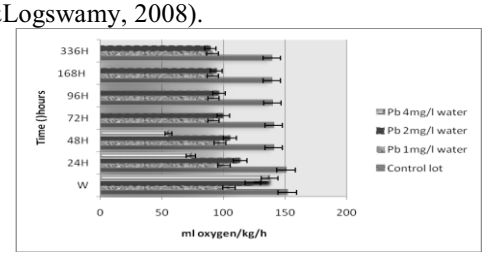


Figure 2. . The influence of the lead upon oxygen consumption on prussian carp (*Carassius auratus gibelio* B.)

The characteristic symptoms of chronic lead intoxication include changes in the blood picture, with severe damage to the erythrocytes and leucocytes, degenerative alterations of the parenchymatous organs and damage to the nervous system. (Výzkumný Ustav Rybářský & Svobodová, 1991)

Haematological studies in fishes have assumed greater significance because these parameters were to be used as an effective and sensitive index to monitor physiological and pathological changes induced by natural or anthropometric factors.( Masopust et al.,2000).

The acute test (96 hours) records 30% mortality in fish variants from the concentration level of 2 mg Pb/l water, and 100% mortality from the concentration level of 4 mg Pb /l water. After two weeks of exposure to the lead concentrations of 1 mg Pb /l water, 2 mg Pb /l water, number of erythrocytes in Prussian carp decrease significantly compared to the control groups (Figure 3). can say that these concentrations produce hemolysis, is impaired erythropoiesis.

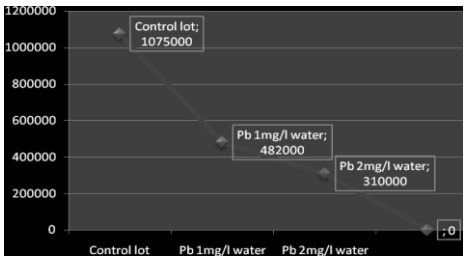


Figure 3. Number of erythrocytes of prussian carp (*Carassius auratus gibelio* B.) for 14 days of exposure lead

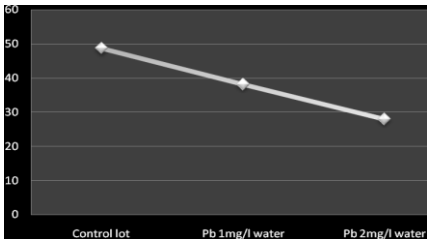


Figure 4. The influence of lead upon glycaemia level on prussian carp (*Carassius auratus gibelio* B )

The glycaemia level in the fish individuals subjected for 14 days to immersion into water with 1mg lead /l water and 2 mg lead/ l water, was also significantly affected as shown in figure 4. After 14 days of immersion the Prussian carp to the lead action in concentrations of 1mg/l and 2mg/l, the glycaemia values are lower than the ones of the controls, indicating a strong impairment of carbohydrate metabolism. A possible explanation for the lead's effect on glycaemia can be its toxic action on liver that represents the haemostatic center of the entire organism, decreasing the amount of glucose released into the blood at this level.

The differences in all parameters physiological are statistically significant ($P > 0.05$) with the concentrations used in experiment.

## CONCLUSIONS

After 14 days of exposing Prussian carps to the lead in concentrations of 1mg Pb /l water, 2mg Pb /l water, 4mg Pb /l water, modified the values of breathing frequency. In the all experimental variants inhibiting of the breathing frequency of fish.

The lead had an inhibitory effect on the energy metabolism of Prussian carps at concentrations of lead.

After 14 days of immersion the Prussian carp to the lead action in concentrations of 1mg/l and 2mg/l, the glycaemia values are lower than the ones of the controls, indicating a strong impairment of carbohydrate metabolism.

After two weeks of exposure to the lead concentrations of 1 mg Pb /l water, 2 mg Pb /l water, number of erythrocytes in Prussian carp decrease significantly compared to the control groups can say that these concentrations produce hemolysis, is impaired erythropoiesis.

The increase of death with increasing concentration and increasing of the duration of exposure could be because of the accumulation of metals in different tissues of body especially in the gills which are important sites for the entry of metals, therefore causing lesions and gill damage and failure of metabolic activities (Bols et al., 2001; James et al., 2003).

## ACKNOWLEDGEMENTS

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✔*Ingineria mediului*

**Vol. XVII ( LIII ) - 2012**

# THE ECO-TOXICOLOGICAL INFLUENCE OF THE PESTICIDE/INSECTICID THIAMETHOXAM OVER SOME VARIETIES OF *CARASSIUS AURATUS GIBELIO BLOCH*

Fodor Marioara[1]

**Keywords**: *eco-toxicity, pesticide, insecticide, fishes*

## ABSTRACT

*The effect of loading the agricultural soil with pesticides and then their reach in waters through infiltration in the groundwater or through soil wash due to rains or controlled discharges of chemical residue in rivers determine disastrous effects under eco-toxicological aspect over aquatic organisms, respectively fishes. In this way the porpoise of this paper is to highlight the eco-toxicological effect of Thiamethoxam pesticide, over some fish exemplars from Carassius auratus gibelio Bloch species by influencing the oxygen consumption, implicitly of the respiratory (energetically) metabolism.*

## INTRODUCTION

Bibliographic data, implicitly the researches in eco-toxicological profile, puts in evidence the harmfulness of the anthropogenic pollutants from the pesticide category, both in terms of toxicological actions and retention in soil, water and organism. In this way is important to remark the process of bio-amplification of the toxic actions of pesticide in the plants and animal body, but especially to human body due to the food chain. With other words, to the human organism is transferred a certain dose of toxicity through the digest of agro-alimentary, which come from plants and animals contaminated with this kind of toxic.

The excess use of pesticide in agriculture puts in discussions the problem of their utility, due to the toxicity and the big degree of retain, even bio-amplification, reason for which the general accepted option is the one that implies their replace with ecological pesticide. These ones are considered the future option, because are based on bio-degradable products, respectively certain fat acids (Botnariuc N. Vădineanu A., 1982, Brezeanu, Gh, Simon - Gruița A., 2002, Cotrău M, Popa L., Stan T., Preda N., 1992).

In this way, the thematic addressed in this paper is very important in our days, representing an eco-toxicological impact study of some pollutant substances, respectively, the Thiamethoxam pesticide, over some exemplar of fishes from the species: *Carassius auratus gibelio Bloch*

Interesting is their adaptive limit, at conditions of water environment contaminated with pesticide, way in which it can be appreciated which is the contribution of science to

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resolving the problem of water eco-system stability. So, in time, as an adaptive and defense reaction to every aggressive form of pollution, the living organisms had elaborated their own response mechanism, nonspecific, mono type, for every toxic substances, some of bio-chemical order, other of physiological-metabolically order etc. (Marinescu Al, 2000, Newman, 2009).

All this have as main purpose the resistance of organism to the diversity of the pollutant factors, in the way of species adaptation and perpetuation. It goes without saying that the species which are more sensible or unadapt disappear and other which are more resistant take their place, for maintaining the ecological equilibrium of the food chain, but also of the living element (Petre M., 2003 Tjeerdema, R., 2012)

## MATERIAL AND METHODS

**The biological material** used for scientifically researches is represented by young exemplars of *Carassius auratus gibelio Bloch* species, which are part of the class Actinopterygii*, subclass: Teleostei, order: Cypriniformes, family: Cyprinidae, kind: Carassius.*

It was picked *Carassius auratus gibelio Bloch* on the base that is the most know and widespread species from Romania, being natural adapted to the most aquatic ecosystems. In this way was appreciated that it is a biological material easy to find, resistant to laboratory analyses: physiological, toxicological, biochemical, cytological, histological, etc.

The exemplars have a large body (important detail for analyses) and adapt easily to laboratory conditions (closed environment – aquariums, artificial food). They are representative for continental waters, covering the ecological valences of organisms: criofile, euriterme, thermophilic, respectively, eurioxibiont. Regarding the life conditions the exemplars are not pretentions, way in which populates waters poor in oxygen from lakes and running waters (Botnariuc N., Vădineanu A., 1982).

*Carassius auratus gibelio Bloch* can be found in all European states and in our country in the majority of rivers. It has a short head, medium size eyes, body covered with large scales, well fixated, length up to 30cm and a maximum weight of 150g. Exceptionally it can reach 1,5…2kg. The fins are dark with orange or red shades. The color of scales depends on the water in which the *Carassius auratus gibelio Bloch* lives, so in muddy waters its scales tend to be black and in those with much vegetation they tend to be green. In clear waters the fish color is silver.
(http://www.crap.ro/pagina/74/305/carasul.html).

It can live in waters which are very poor in oxygen with different limits of the PH, being resistant even if is taken out of the water (several hours), but also to water excessive heating to a temperature of 40°C in which it can be found a minimum quantity of oxygen. It feeds with larvae, crustaceans, plants, mollusks, eggs etc.
(http://www.wikipedia.ro).

The biological material was represented by young exemplars with medium lengths, healthy, with non-parasites.

**Used method: WINKLER** – for the determination of the oxygen dissolved in water and the calculus of the respiration metabolism to exemplar of fishes subject to the analyze (*Carassius auratus gibelio Bloch*) Mălăcea I. 1969, Strungaru, Gr., Pop, M., Hefco, V., 1983 Marinescu, Al. G., 2000).

Was used:

1. Alkaline solution of potassium iodide (KI + NaOH)
2. Solution of manganese chloride ($MnCl_2$)
3. Solution of sodium thiosulphate 0,01 N ($Na_2S_2O_3$)
4. Concentrated hydrochloric acid (HCl)
5. Solution of starch 1%
6. Sulfuric acid 10%:
7. Starch glue 0,2

## RESULTS AND DISCUTIONS

For the experimental variant of intoxication with the Thiamethoxam pesticide (insecticide) were made two lots:

●***Lot 1*** *– in which were used 7 exemplars of fishes from Carassius auratus gibelio Bloch specie with an average weight of 17 g expose to the action of* Thiamethoxam, in concentration of *3 mg/l water*.

●**Lot 2** - *similar with 7 exemplar of fishes from Carassius auratus gibelio Bloch specie was introduced in clean water to have a witness mark – M, which has approximately the same average weight, namely 17,4 g*

To make a double verification of the witness mark (M), even the Lot 1 was introduced before the pesticide application, for 24 hours, in clean water and then was calculated the oxygen consume. The obtained values were very close to (M) or with insignificant differences.

Were made preliminary tests regarding the survival degree of the *Carassius auratus gibelio Bloch* exemplars, but also to different applied toxic doses. In this way, was established the interval until lethality. The laboratory studies were made at temperatures between 17–20 $^0$C and the fishes were not feed during experimentations. The experiments focused ethological investigations, determination of the oxygen consume and the calculus of the respiration movement frequency.

►**Ethologic aspects**: The all exemplar presented interest under behavioral aspect, their manifestation being similar. The first behavioral signs in conditions of intoxication with Thiamethoxam were agitation, restlessness, rapid movements, repeated lifting to the water surface, reflected through the excessive oxygenation tendency (increased number of opercular movements). The second stage was characterized through the increase of the rapid movement. The third stage was characterized by the appeasement status of the exemplars, with slow movements and reflexes. Towards the end were observed balancing body movements, torsional, vertical or oblique position, after which followed death. All this ethologic aspects confirmed the theoretical data from specialized literature. Interesting were some initial adaptive reactions to the toxic dose.

►**Metabolic aspects:** The determinations of the oxygen consume for the exemplars of Lot 1 (calculated through *Winkler method*) were done independently, for each individual in part. So for the 1 and 7 exemplars the experimental data are presented in table 1 and table 2 .

**No. 1 exemplar**

a) fish weight: **17 g**

b) average oxygen consumption in clean water **147.03 ml/kg/hour (M)**

c) respiratory frequency : **75**

Table 1

**No. 1 exemplar**

| No crt | G(g) | M | | first day | | II day | | III day | | IV day | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 17g | CO | 147,2 | CO | 220,54 | CO | 146,9 | CO | 73,5 | CO | 72,9 |
| | | F | 75 | F | 36 | F | 57,3 | F | 86 | F | 84,66 |

CO – oxygen consumption, represents the intensity of the energetic metabolism ($mlO_2$/Kg/h).

F – respiratory movement (opercular) frequency, namely the number of the opercula movement during one minute observed on each exemplar taken from the lot and put in a separate recipient for 15 minutes. The first 10 minutes are necessary for calming. Afterwards are done three successive verification and an average value is calculated. With other words F is an average respiratory frequency.

Data is compared with witness (M).

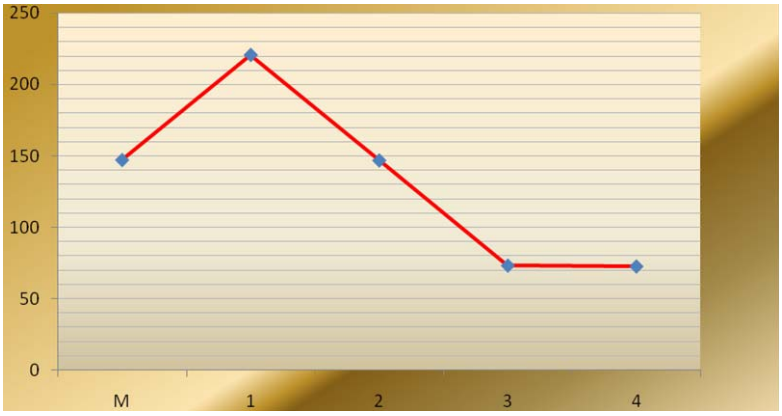


Figure 1. *Variation of oxygen consumption to the first exemplar of Lot 1 under the influence of Thiamethoxam, c% 3 mg/lwater*

**No. 7 exemplar**

a) fish weight: **14 g**

b) average oxygen consumption in clean water **147.03 ml/kg/ hour (M)**

c) ) respiratory frequency: **74.16**

Table 2

**No. 7 exemplar**

| Nr crt | G (g) | M | | first day | | II day | | III day | | IV day | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7. | 14g | Co | 157,04 | Co | 193,79 | Co | 120,28 | Co | 83,52 | CO | 83,8 |
| | | F | 74,16 | F | 46 | F | 71,49 | F | 89,5 | F | 89,9 |

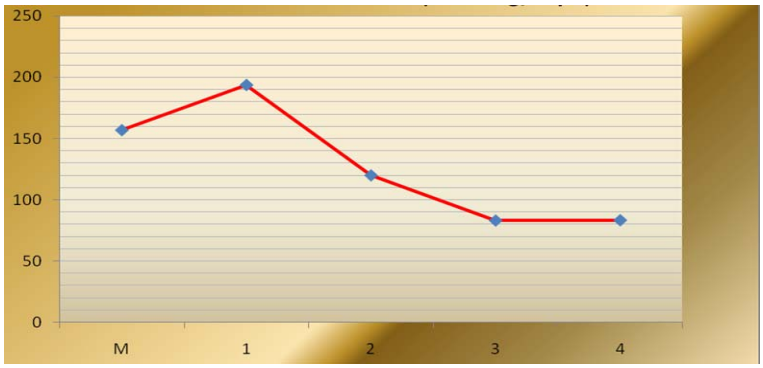


Figure 2. . Variation of oxygen consumption to the seventh exemplar of Lot 1 under the influence of Thiamethoxam, c% 3 mg/lwater

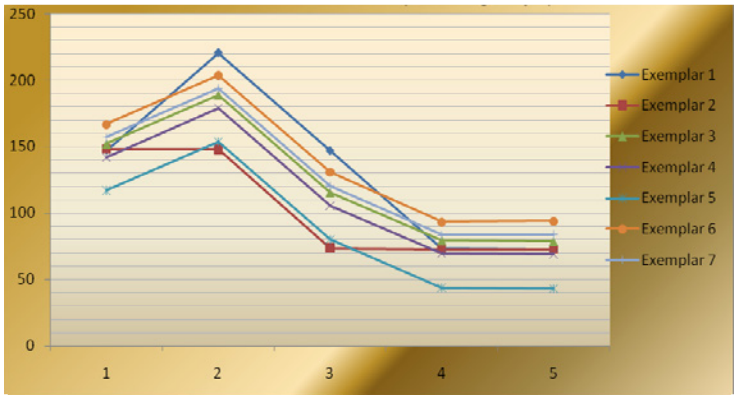


Figure 3. Graphic representation of the oxygen consume to all 7 exemplar of Carassius auratus gibelio Bloch intoxicated with Thiamethoxam in concentration of 3 mg/l water

## CONCLUSIONS

The all exemplars have common reactions during the entire experiment, ethological and physiological referring to the oxygen consumption and the respiratory metabolism.

It is observed than under the action of the toxic Thiamethoxam insecticide, in concentration of 3 ml/l, between the first and second day exists an increase of the oxygen consumption to all seven exemplars of *Carassius auratus gibelio Bloch*

It is observed that in the interval 3- 4 days the oxygen consumption decreases very much reaching even a minimal value after which increases barely, insignificant, on an ample bearing.

The graphic of the seven exemplars put to laboratory analyzes are similar from the point of view of the representative segments.

This fact attests the validity of the experiments and the similar behavior of the researched exemplars and it can be said that they have a similar metabolism rate. Afterwards the all three segments from the figures are descendant having smaller values of the oxygen consume.

This descendent tendency is common for the all seven exemplar, fact which validates the experiment.

The oxygen consumption decrease is potentiated and by the lack of food during experiments.

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**Vol. XVII ( LIII ) - 2012**

# TOXICOLOGICAL RESEARCH OVER SOME VARIETIES OF CARASSIUS AURATUS GIBELIO BLOCH, UNDER THE INFLUENCE OF COPPER SULPHATE

Fodor Marioara[1]

**Keywords**: *eco-toxicity, copper sulphate, heavy metals, fishes*

## ABSTRACT

*In this paper is put in evidence the eco-toxicological effect of the copper sulfate ($CuSO_4$), through the use of biological material of some varieties of Carassius auratus gibelio Bloch species. Interesting from a scientifically point of view is the symptomatically picture of the intoxicated fishes, the lethal doses and the adaptive reactions to toxicity.*

*Also, the intoxication with metallic pollutant substances and their complex combinations, can induce even the sterility of the living organisms, respectively, weakening the perpetuation capacity of species, reaching sometimes the destruction of some populations which are less resistant to the action of the harmful element or which can't adapt to new environment condition. As result, the stability of most ecosystems decreases, the productivity and the population genes stock modifies, taking place irreversible transformations.*

## INTRODUCTION

The presented case, respectively to one of the toxicologically influence of some, heavy metals and their salts, over some species of sweet water fishes, is wanted to be representative regarding the amplitude of the atrophic pollution phenomena.

The toxicity which is developed at general level, in every living environments, both at the level of the inferior organisms and the superior ones, from which take part the humans, induces multiple modifications of every type: somatic, physiologic-metabolic, bio-chemical, cytological, hematological, neurological, genetically etc.

In this conditions, *the adaptive capacity* of living organisms is put to test, through specific defense reactions. In this way, sometimes these are represented by complex biological reactions, due to is induced the possibility of disintegration and neutralization of many toxics or by *physiological reaction* implicitly adaptive metabolic, breathing, secretor (mucus in the fish case), *ethological* (avoiding the zone were the presence of toxic is sensed) etc.

Is possible that this adaptive reactions to give a survival note in diverse conditions, unfavorable and to generate specific modalities to save the species.

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But this thing must not lead to the idea of saving the organisms through themselves, but more through uniting all human forces with the purpose of preventing and counteraction all polluting form, event the atrophic ones. This is the true possibility to save the biosphere.

With other words, in an environment problem, there must be put in evidence the circuit and the toxic effect. To be taken into account the pollution source, the eco-toxicological effect over the environment (namely the aquatic one).

Finally to observe the toxic effect over species of fishes put to the analyses.

Are important all the experiments of any kind: physiological, cytological, bio-chemical, hematological, genetically, of toxicological analyses of heavy metals, of retaining in fish organism.

The toxicological evaluation must take into account, firstly, the harmful, negative or even lethal effect, over the living organism, of any kind, but also of the possibility of its accumulation, amplification and transformation (Petre M., 2003, Râpeanu M., 1983, UE, 2006, Moore, J., Ramamoorthy S., 2012)

## MATERIAL AND METHODS

The biological material used for scientifically researches is represented by young exemplars of *Carassius auratus gibelio Bloch* species, which are part of the class *Actinopterygii, subclass: Teleostei, order: Cypriniformes, family: Cyprinidae, kind: Carassius.*

The biological material was represented by young exemplars with medium lengths, healthy, with non-parasites. In this way was appreciated that it is a biological material easy to find, resistant to laboratory analyses: toxicological, physiological.

Among the methods used, there are the chemical ones, for obtaining the dilutions, respectively the solutions of $CuSO_4$, the biological ones, for analyzing the varieties subject to experiments, the ones of direct observation, and the ethological (behavioral) ones.

## RESULTS AND DISCUTIONS

Among the methods used, there are the chemical ones, for obtaining the dilutions, respectively the solutions of $CuSO_4$, the biological ones, for analyzing the varieties subject to experiments, the ones of direct observation, and the ethological (behavioral) ones.

The experiments were made on *Carassius auratus gibelio Bloch,* setting up 6 lots of 7 varieties each, which were dosed with various concentrations of copper sulfate *($CuSO_4$),* respectively, 0.01, 0.03, 0.08, 0.1, 0.4.

During the experiment, for the validation of the results, the concentrations of $CuSO_4$ or the administered dosages were taken into account.

The conditions in which the fish lots were kept were optimum. A special attention was granted to constantly maintaining the values of some water parameters, such as: temperature, pH and dissolved oxygen, so that their modifications might not amplify the toxic action of the copper sulfate.

The beginning of lethality was registered at the concentration of 0.08 mg $CuSO_4$/l water, at 24 hours, the mortality rate being of 20% at this lot.

The maximum mortality of 70% was noticed at the concentration of 0.4 mg/l $CuSO_4$ at toxic exposure of 96 hours.

At the dosage of 0.08 mg/l $CuSO_4$, the lethality increases in direct proportion with the exposure time.

The mortality rate varies depending on the concentration of the substance and on the exposure time at the toxic substance.

The fish behavior during the experiments was compared to the witness lot (M), which was kept in clean water.

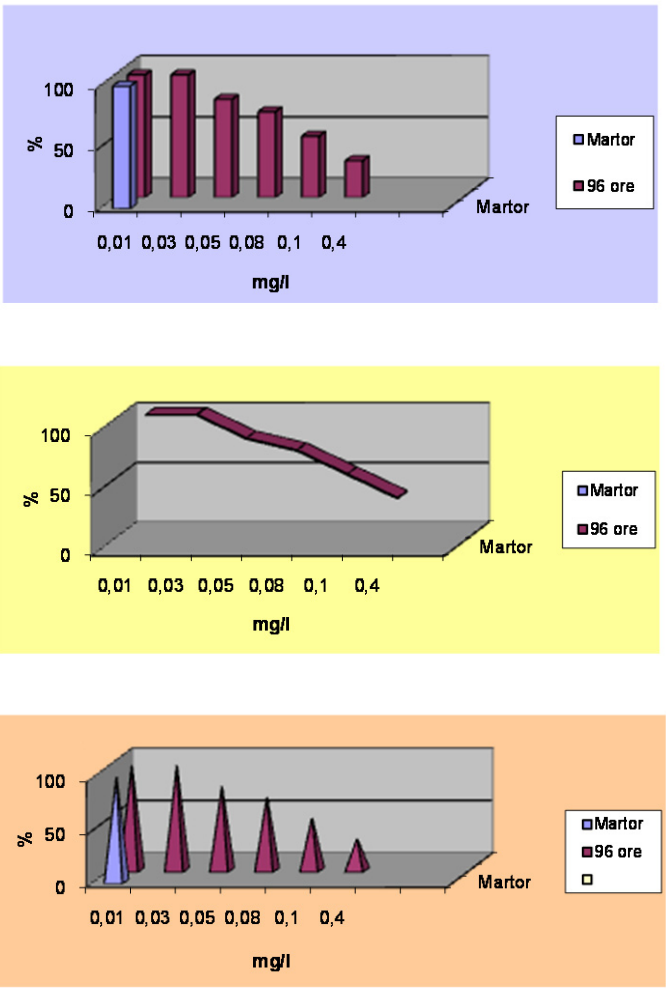


Fig. 1,2,3 Graphic representation of the survival/mortality percentage of varieties of *Carassius auratus gibelio Bloch* species, after a 96 h exposure with different concentration of $CuSO_4$

After 96 hours, we record a mortality of: 30% from the varieties, for 0.08% $CuSO_4$; 50% at 0.1 mg/l $CuSO_4$; 70% for 0.4 mg/l $CuSO_4$.

**The table with the symptoms of the fish intoxicated with copper sulfate**

Inquietude stage – after the contact with the toxic substance, fish become visibly agitated, making rapid movements (represented with the symbol:○). The frequency of the fish getting up at the surface of the water increases and their swim becomes very agitated, jumping a lot (jumps have the symbol↯), and their body shivers.

The amplitude of their manifestations is depending on the concentration of the toxic substance administered (0.01% $CuSO_4$), but also on the exposure time.

Increase of irritability – the varieties manifest agitation (represented with:○), inquietude and respond with characteristic sudden movements (at a dosage of 0.01-0.03% $CuSO_4$).

Tranquility stage–it is significant for all the tested varieties, after the agitation from the first two stages. They present slow swimming, having ampler movements and very slow reflexes (0.03% $CuSO_4$).

Balance troubles – represented by slow swinging movements of the body or twists. Sometimes, the body position is horizontal, sometimes it may be vertical or oblique.

These manifestations are specific for the varieties exposed at concentrations of 0.08 and 0.1 mg/l $CuSO_4$ being associated with dorsal decubitus (↓ falling on their back), and lateral decubitus (→ falling on one side ) and loss of the capacity to react at mechanical stimuli.

This stage is characterized by abundant secretion of mucus, which is at first accumulated on their gills, and, later on, on their entire body. Also, a phenomenon of exophthalmia was noticed.

Some of the fish, treated with 0.1 mg/l substance, presented edema or bleeding of branchial epithelium and oral cavity, degeneration and partial detachment of the mouth mucous and even of pharyngeal epithelium.

Registration and interpretation of the symptoms presented by the fish may offer us important indications regarding the action of the toxic (copper sulfate) on them. Thus, the apparition of cramps and paralysis involves nervous dysfunctions.

Paralysis of the dorsal fin and of the pelvic fins, peripheral movement disturbances and also of the respiratory organs, which induce asphyxia.

Deterioration of the gills lead to asphyxia, due to the concentration / 0.1-0.3% of $CuSO_4$, by generation of plasmolysis of the branchial epithelial cells. Increase of the respiration frequencies (metabolic effect of the lack of oxygen) is a different ethological (behavioral) reaction.

This is due to the damaging of the gills, which lead to the decrease of their functional capacity and to the diminution of the intake of oxygen.

Pigmentation of the skin or its discoloration are signs of nervous intoxication or of some problems of the endocrine system, fact noticed at some varieties (Fig.4, photo no.1).

A very frequent symptom is asphyxia, accompanied by the increase of breath frequency, which forces the fish to rise to the surface of the water, to get air. These symptoms are followed by a slowing of movements, paralysis and ataxia. In this regard, we may say that there is a combined effect of the lack of oxygen and of the accumulation of carbon dioxide in the blood.

Due to the accumulation of the carbon dioxide, breathing is damaged, due to the loss of excitability of the respiratory system, which leads to progressive paralysis, mainly of the heart activity. In case of hypoxia, fish keep their mouth wide open, and opercula are very wide. When asphyxia occurs, due to the accumulation of carbon dioxide in the blood, fish keep their mouth half-open, and opercula are slightly open.

Fig.4 Photography no.1 of a *Carassius auratus gibelio Bloch* exemplar which has pigmentar spots or tegument discoloration, as a result of copper sulfate intoxications (Orig.)

Total ataxia – is the stage when fish lose completely their balance and fall in lateral or dorsal decubitus, making unsuccessful attempts to get their pose. The lots of fish exposed to big concentrations had rarer operculum movements, thus appearing exhaustion - $CuSO_4$ (0.1 – 0.3%).

Agony – occurs in an advanced degree of intoxication, when fish are on the bottom of the aquarium, not being able to move. The breathing movements are very weak - $CuSO_4$ (0.3 – 0.4%), (Fig. 3 / photo 2).

Death – is the last stage, followed by cadaveric rigidity (Fig. 5 / photo 2).

Fig. 5. Photography no. 2 of two exemplar of *Carassius auratus gibelio Bloch:* one in agony phase the other in lethal phase (Orig.)

Among the most dangerous heavy metals, we mention: Cd, Pb, Hg, Cr, Zn, Co, Ni, As, Fe, Sn, Sb, Au, Ag, Cu, Vn, Sn, even if some of them are deemed vital for our body (however, in very small concentrations), but become toxic in larger concentrations.

## CONCLUSIONS

The factors of the aquatic environment influencing the toxicity of the heavy metals as well as their complex combinations are: temperature, content of oxygen, composition of salts, pH, etc. The study of the degree of toxicity of the heavy metals has led to the idea that the toxic action of the ions is caused by the easiness in which they form compounds with the proto-plasmatic substances, respectively with proteins.

Fish defend themselves from toxic substances by abundant secretion of mucus, the modification of the enzymatic systems, so that to allow the metabolization of some toxic substances and their transformation into non-toxic products.

Accommodation at high concentrations of toxic substances leads to the accumulations in tissues.

The varieties subject to analysis have the capacity to feel, though their senses, the alteration condition of the aquatic environment, therefore they become agitated.

Lethal limits at the varieties subject to the analysis for copper are between 0.03 – 0.4 mg/l.

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# AGROBIOLOGICAL AND TECHNOLOGICAL CHARACTERISTICS OF INTERSPECIES NEWLY SELECTED WINE GRAPE VARIETIES IN THE INSTITUTE OF VINE AND WINE PLEVEN

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**Keywords:** *vine, interspecies varieties, agrobiological and technological characteristics*

## ABSTRACT

*Translated is agrobiological and technological study of 2 white (Glory and Pleven dew) and 2 red (ruby Trapezitsa and Kailashka) newly selected interspecies wine grape varieties. These varieties are characterized by a high degree of fertility, increased resistance to low winter temperatures and mildew and Oidium, which guarantees them an annual obtaining high yield and grape quality. The values of the parameters of the mechanical analysis of grape and grain boundaries in a typical wine grape varieties. Wines obtained are characteristic of the variety flavor.*

## INTRODUCTION

For Bulgaria, the vine is one of the most valuable crops. A prerequisite for this is the favorable soil and climatic conditions and experience in growing it. In this zone, and in our country are harvested hundreds of varieties that differ in their morphological characteristics, biological and technological properties. Despite the great variety of cultural diversity Vine / *Vitis vinifera L.* /, most of them exhibit increased sensitivity to low winter temperatures and major diseases and pests on the vine. This is a limiting factor for their widespread in the world and at home.

Experience of the world science shows that the use of selection-genetic methods for obtaining varieties with increased resistance to stress factors emerged as the most reliable, most cost-efficient and safe for human health method (Alleweldt & Possingham, 1988). Selection-genetic research for sustainable varieties in Bulgaria began in the 60s of last century IVW - Pleven. It is built on a large scale and to date has achieved significant results (Valchev 1978, 1990; Zankov, 1974, 1975; Ivanov Valchev, 1971).

The aim is to study for Agrobiological and technological study of in novoselektsioniranite IVW - Pleven wine grape varieties with increased resistance to stress factors: Slava, Pleven dew (white) and Trapezitsa, Kailashka ruby (red).

## MATERIAL AND METHOD

The experimental work was carried out during the period 1994 - 2004, in the experimental base of IVW - Pleven. From the examined varieties are planted 40 vines each.

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The vines are over 10 years old, grafted on rootstock Riparia x Berlandieri ML4 stem and are grown on a leveled formations Moser load. The studied varieties defined indicators and actual fertility is performed mechanical analysis of grapes and grain (Katerov and others. 1990).

Of the newly selected interspecies wine grape varieties vines was investigated degree of cold tolerance under field conditions in the year in which they are registered crucial for the development of low winter temperatures (1993 -21,5 º C, 1995 -23,0 º C, 1997 -23, 4 º C), it was recorded as the percentage of damaged main and replacement buds in winter buds. Used for the control of wine grapes *Vitis vinifera L.* - Cabernet Sauvignon, Pamid and Dimyat.

During the vegetation period newly selected interspecies wine grape varieties were evaluated degree of resistance to the ordinary mildew and (*Plasmopara viticola* / Berk & Curt.) Under field conditions to control - varieties of *Vitis vinifera* L. - Chardonnay and Cabernet Sauvignon. Under field conditions, the results are reported using the Husfeld (1933).

Of each variety in the Research winery of the Institute is vinified in 30 kg of grapes in the traditional technology of red and white wines (Yankov and others., 1992; Amerine et al., 1972). Grape must (in white) and grape pulp (in red) is sulphited 50 mg/dm3 SO2. Alcoholic fermentation of experimental variants was carried out with a clean dry wine yeast culture Saccharomyces serevisiae in the amount of 10 g / hl, at 20 ˚ C (white) and 25 to 26 ˚ C (red) in daily control. Young white and red wines made physico-chemical analysis of the main indicators in conventional winemaking methods (Ivanov and others, 1979). Organoleptic properties were evaluated in 100-level score (Tsvetanov, 2001) by an expert tasting committee.

## RESULTS AND DISCUSSION

Average for the period of the study, studied wine grape varieties are characterized by high levels of actual fertility (Table 1).

In white wine varieties percentage of their winter eye is relatively high and 71.03% in cultivar Slava and 76.28% in Pleven dew, and the developing fetus shoots respectively 79.42% and 72.63%. In red wine varieties percentage of developed shoots is high and 78.98% in Trapezitsa and 86.82% in Kailashki ruby. From the study it is found that the average period for a variety Trapezitsa most shoots have developed fruit - 77.04%, while the variety Kailashki ruby they are significantly less - 70.82 percent. The ratio of fruit abundance of all species studied is very high - 1.53 (Trapezitsa), 1.79 (Slava), 1.83 (Kailashki ruby) and 1.96 (Pleven dew).

Table 1

Actual yield of wine grape varieties examined average for the period 1994-2004

| Variety | Buds left | Developed buds | Fruiting shoots | Coefficient of fruit abundance |
|---|---|---|---|---|
| | pieces | % | % | |
| **White wines** | | | | |
| Slava | 40,00 | 71,03 | 79,42 | 1,79 |
| Pleven dew | 40,00 | 76,28 | 72,63 | 1,96 |
| **Red wines** | | | | |
| Trapezitsa | 40,00 | 78,98 | 77,04 | 1,53 |
| Kailashki ruby | 40,00 | 86,82 | 70,82 | 1,83 |

Table 2

Mechanical analysis of grapes and grain from the examined white and red wine grape varieties on average for the period 1994-2004

| Variety | Average mass of the grapes | Size of the grapes | | Average weight of 100 grapes | Size of the grains | | Mechanic analysis | | | | | Chemical analysis | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Grape | | Grain | | | | |
| | | Length | Width | | Length | Width | clusters | grains | skins | seeds | Mezzo carp | sugars | titratable acids |
| | g | cm | cm | g | mm | mm | % | % | % | % | % | % | g/dm$^3$ |
| **Wine wines** | | | | | | | | | | | | | |
| Slava | 106,0 | 14,1 | 6,8 | 162,0 | 12,10 | 11,70 | 3,90 | 96,10 | 9,80 | 5,00 | 85,20 | 20,40 | 7,40 |
| Pleven dew | 185,0 | 12,5 | 8,5 | 265,0 | 15,20 | 15,02 | 3,41 | 96,59 | 9,92 | 2,66 | 87,42 | 20,50 | 5,60 |
| **Red wines** | | | | | | | | | | | | | |
| Trapezitsa | 168,3 | 15,1 | 8,3 | 185,3 | 16,88 | 13,75 | 5,26 | 94,74 | 8,34 | 4,31 | 87,54 | 21,70 | 5,27 |
| Kailashki ruby | 203,6 | 14,6 | 9,2 | 177,3 | 13,88 | 13,63 | 4,35 | 95,65 | 10,15 | 4,37 | 85,48 | 22,50 | 7,09 |

Table 5

Physical and chemical analysis of grapes and grain from the examined white and red wine grape varieties on average for the period 1994-2004

| Variety | Alcohol | Sugars | Titratable acids | Volatile acids | Wine acid | Apple acid | Total extract | An. | I | T | pH | ОФ | Degustation evaluation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | об. % | g/dm$^3$ | g/dm$^3$ | g/dm$^3$ | g/dm$^3$ | g/dm$^3$ | g/dm$^3$ | mg/dm$^3$ | | | | g/dm$^3$ | |
| **White wines** | | | | | | | | | | | | | |
| Slava | 11,42 | 1,40 | 7,00 | 0,54 | 2,14 | 1,41 | 20,20 | - | 0,02 | - | 3,14 | 0,33 | 76,55 |
| Pleven dew | 11,49 | 1,37 | 5,40 | 0,42 | 1,64 | 4,42 | 18,10 | - | 0,02 | - | 3,51 | 0,36 | 76,67 |
| **Red wines** | | | | | | | | | | | | | |
| Trapezitsa | 12,15 | 1,74 | 4,65 | 0,60 | 1,86 | 3,75 | 24,70 | 224 | 6,53 | 0,63 | 3,25 | 2,20 | 75,30 |
| Kailashki ruby | 12,60 | 1,95 | 6,15 | 0,60 | 1,37 | 4,99 | 25,90 | 286 | 7,26 | 0,57 | 3,49 | 1,18 | 76,83 |

The average mass of a cluster in the studied varieties is from 160,0 g in variety Slava to 203,6 g in variety Kailashki ruby (Table 2). The dimensions of the cluster are 12,5 / 8,5 cm in variety Pleven dew to 15,1 / 8,3 cm in variety Kailashki ruby. The average weight of 100 grains ranges from 162,0 g in variety Slava to 265,0 g in variety Pleven dew. Grain sizes are 12,10 / 11,70 mm in variety Slava to 16,88 / 13,75 mm in variety Trapezitsa. As these indicators are typically studied varieties wine with specific characteristics of grapes and grain. Made by mechanical analysis showed that the percentage of grains in the studied varieties is high and 94.74% in variety Trapezitsa to 96.59% in variety Pleven dew. The skin is more variety in Kailashki ruby - 10.15%, the seed variety for Slava - 5.00% and in variety mezocarp Kailashki ruby - 80.70%.

The study of wine grape varieties resistant to ordinary mildew (*Plasmopara viticola* / Berk & Curt.) is one of the many important tasks in grapevine breeding work. At the interspecies newly selected wine grape varieties during the study were carried out under field conditions for resistance to common blight.

During the whole period of study control varieties Chardonnay and Cabernet Sauvignon confirm its strong susceptibility to the ordinary mildew and - State 5 (Table 3). Variety Pleven dew and Trapezitsa resistant to slightly susceptible (2-3 bale). With relatively good resistance to ordinary mildew during the study were characterized varieties Kailashki Ruby and Slava - 2 ball (sustainable). It should be noted that in some years when climatic conditions were not appropriate for the development of the disease, the newly selected interspecies grape varieties showed complete resistance to the ordinary mildew.

Table 3

Resistance of newly selected interspecies wine grape varieties to the ordinary mildew (*Plasmopara viticola*/Berk & Curt.)

| Variety | Resistance in balls |
|---|---|
| **White wines** | |
| Chardonnay | 5 - ball /highly susceptive/ |
| Slava | 2 – ball /resistant/ |
| Pleven dew | 2 - 3-ball /resistant to less susceptive/ |
| **Red wines** | |
| Cabernet sauvignon | 5 - ball / highly susceptive / |
| Trapezitsa | 2 – 3- ball / resistant to less susceptive / |
| Kailashki ruby | 2 - ball / resistant / |

Of the examined of red and white wine varieties interspecies has been done a research regarding the degree of resistance to low winter temperatures. Data are presented in Table 4.

Analysis of the data relating to the extent of damage from low winter temperatures under field conditions show that the standard varieties of *Vitis vinifera L.* - Cabernet Sauvignon and Pamid Dimyat, winter eyes are much more affected by these con-wine varieties. Despite the reported injuries on the head and replacement buds studied varieties is produced normal yield grapes of good quality, and control varieties of *Vitis vinifera L.* quantity of grapes is less and lower quality.

Average quantities of sugars and titratable acidity strongly influenced by meteorological conditions during the different growing seasons, especially during the ripening of the grapes.

Data analyzed physico-chemical analysis of grapes show conditioning of the must suitable for the production of white and red wines. In white wine varieties the sugar content is 20.4% in variety Slava and 20.5% in Pleven dew and titratable acidity, respectively 7,4 g/dm3 and 5,6 g/dm3. In red wine grape sugar content in grapes is 21.7% in Trapezitsa and 22.5% in Kailashki ruby and acids respectively 5,27 g/dm3 and 7,09 g/dm3 (Table 2).

Table 4

Degree of resistance newly selected interspecies wine grape varieties to low winter temperatures

| Variety | Main buds | | Replacement buds | |
|---|---|---|---|---|
| | Healthy | Dead | Healthy | Dead |
| | % | % | % | % |
| **1993 (t° = -21,5°C)** | | | | |
| Cabernet sauvignon /control/ | 9,78 | 90,22 | 15,22 | 84,78 |
| Slava | 98,80 | 1,20 | 100,00 | 100,00 |
| Kailashki ruby | 91,43 | 8,57 | 91,43 | 8,57 |
| **1995 (t° = -23,0°C)** | | | | |
| Dimyat / control / | 30,00 | 70,00 | 44,15 | 55,85 |
| Slava | 100,00 | 00,00 | 100,00 | 00,00 |
| Pleven dew | 97,75 | 2,25 | 99,44 | 0,56 |
| Trapezitsa | 96,91 | 3,09 | 99,48 | 0,52 |
| **1997 (t° = -23,4°C)** | | | | |
| Pamid | 3,26 | 96,74 | 42,39 | 57,61 |
| Trapezitsa | 22,44 | 77,56 | 65,38 | 34,62 |
| Kailashki ruby | 16,78 | 83,22 | 59,06 | 40,94 |

From the data presented in Table 5 shows that grapes from varieties study failed to mature in the soil and climatic conditions of the city of Pleven.

Received wines are characterized by the following main features:

Slava variety of wine is characterized by a yellow-green color, pleasant floral aroma. Taste the wine is soft, gentle, good freshness and harmony.

In Pleven dew wine variety with yellow-green color, expressed muscat flavor, freshness characteristic of the variety and harmony.

Trapezitsa variety of wine has a nice, ruby red color and well pronounced fruity aroma. Taste the wine is soft and harmonious.

Wine of Kailashki ruby variety features deep ruby color with a pleasant aroma, extractive, thick, harmonically.

Good characteristics of wines complement and healthy grapes and climatic conditions of the region.

The ongoing annual wine tasting test of trial tasting varieties received high ratings. Differences in average estimates of the blame for the entire period of study are small - Pleven dew - 76.67, Slava - 76.55 Trapezitsa - 75.30 and Kailashki ruby - 76.83 Ball.

## CONCLUSIONS

The average values of the parameters of the actual fertility indicate that examined varieties with higher yield, which guarantees annually to obtain a high yield from quality grapes.

Data from mechanical analysis show that these are typical grape varieties with specific characteristics of the grape and grain, suitable for the production of quality red and white wines.

During the whole period of study under field conditions, as resistant to slightly susceptible to the most economically important disease in vine - ordinary mildew occur interspecies grape varieties Pleven dew and Trapezitsa. With greater resistance varieties are characterized Kailashki Slava and ruby. European varieties Chardonnay and Cabernet Sauvignon confirm its strong susceptibility to the ordinary mildew.

The degree of damage to the main and substitution buds in winter buds from low winter temperatures under field conditions the examined interspecies wine grape varieties for cold tolerance significantly outperform standard grape varieties Cabernet Sauvignon, Pamid and Dimyat. In years with critically low temperatures interspecies wine grape varieties is produced in normal quantity and quality of grape production, confirming their genetically determined capacity for higher regenerative capacity.

Obtained wines have very good analytical and organoleptic qualities, featuring characteristic of the variety flavor.

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